THE EFFECT OF SEED TEMPERING AND MICRONIZATION TEMPERATURE ON THE PHYSICOCHEMICAL PROPERTIES OF CHICKPEA FLOUR AND ITS PERFORMANCE AS A BINDER IN LOW-FAT PORK BOLOGNA

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ABSTRACT

The overall goal of this research was to investigate the effect of seed tempering moisture and micronization temperature on the physicochemical properties of chickpea flour and its subsequent performance as a binder in a model low-fat pork bologna product. This work was divided into three studies. In the first study, the effect of seed tempering moisture (untempered (7% moisture) or tempered to 15 or 22% moisture) and surface micronization temperature (115, 130, 150 or 165°C) and on the physical, chemical and functional properties of chickpea flour were investigated. Chickpea flour became darker as seed moisture or micronization temperature increased. Increasing the micronization temperature at 22% seed moisture increased starch gelatinization from 8.2 to 34.0%. The lipoxygenase activity of chickpea flour also was reduced by micronization of seed. Lipoxygenase activity in flour from non-micronized seed and flour from seed micronized at 115°C without tempering was determined to be 1.98×10^5 and 1.12×10^5 units/g of protein, respectively, with no activity found in any other treatments. There was an increase in the water holding (WHC) and oil absorption capacity (OAC) of flour when chickpea seed was tempered to 22% moisture before micronization. Flour from untempered seed and from seed tempered to 15% moisture exhibited small increases in WHC as micronization temperature increased. Micronization had no effect on the OAC of untempered flours, whereas OAC decreased in flour from seed tempered to 15% moisture at higher micronization temperatures. Rapid visco-analysis (RVA) revealed that peak viscosity and final viscosity of all flours from tempered seed decreased with increasing micronization temperature, whereas the trend for both peak viscosity and final viscosity was in the opposite direction with untempered seed.

The effect of seed tempering moisture and micronization temperature on the performance of chickpea flour as a binder in a low-fat, comminuted meat product (i.e., low-fat bologna) was investigated in study 2. Both the textural and sensory properties (trained sensory panel, n=12) of the bologna (10% fat) were explored. In study 3, a consumer panel was performed with 101 untrained participants evaluating selected formulations in order to better understand consumer purchasing behaviour as it relates to comminuted meat products containing a pulse-based binder. Bologna containing flour from micronized chickpea was more yellow in colour (CIE system, trained panel and consumer panel evaluation) compared to those with added wheat flour or no binder. There was no effect of tempering or micronization conditions on cook loss or expressible moisture of bologna containing chickpea flour, whereas bologna produced with wheat flour had the greatest WHC among all bologna treatments. Texture profile analysis (TPA) showed that the addition of chickpea flour from seed tempered to 15% or 22% seed moisture and micronized to 115, 130 or 150^{o}C or flour from untempered seed micronized to 130 or 150^{o}C led to an increase in hardness to a level similar to that of bologna containing wheat flour; sensory evaluation by the trained panel did not produce a similar result. A difference in flavour intensity was not found among all bolognas containing chickpea flour during sensory evaluation. Bologna produced with chickpea flour from seed micronized to 150^{o}C and from seed tempered to 22% moisture and micronized to 115^{o}C was comparable to bologna containing wheat flour with respect to overall texture, overall juiciness and flavour acceptability. These results demonstrated that selection of appropriate seed tempering conditions and micronization temperatures is important with respect to the utilization of chickpea flour as a binder in low-fat bologna.

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LIST OF ABBREVIATIONS

a*	Redness
AACC	American Association of Cereal Chemists
ADF	Acid Detergent Fiber
A-K	Allo Kramer
b*	Yellowness
CIE	International Commission on Illumination
cP	Centipoise
CV	Coefficient of Variation
D	Starch Digested by Amyloglucosidase
DNA	Deoxyribonucleic Acid
DSC	Differential Scanning Calorimetry
EA	Emulsion Activity
EDTA	Ethylene Diamine Tetra Acetic Acid
EM	Expressible Moisture
ES	Emulsion Stability
g	Gravitational Force
G	Gelatinized Starch
g/mol	Gram per Mole
IDF	Insoluble Dietary Fibre
Jg-1	Joules per Gram
K	Kabuli
kDa	KiloDalton
kPa	Kilopascal
L*	Lightness
LFPB	Low-Fat Pork Bologna
LGC	Least Gelation Concentration
LOX	Lipoxygenase Activity
LSD	Least Significant Difference
MW	Molecular Weight

M115	Chickpea Flour from Seed Micronized to Reach 115^{o}C
M130	Chickpea Flour from Seed Micronized to Reach 130^{o}C
M150	Chickpea Flour from Seed Micronized to Reach 150^{o}C
M165	Chickpea Flour from Seed Micronized to Reach 165^{o}C
N	Newton
NDF	Neutral Detergent Fiber
N/g	Newton per Gram
OAC	Oil Absorption Capacity
P	Probability
r	Correlation Coefficient
rpm	Revolutions per Minute
RVA	Rapid Visco Analyzer
RNA	Ribonucleic Acid
RVU	Rapid Visco Units
S	Svedberg Unit
SAS	Statistical Analysis System
SD	Standard Deviation
SDF	Soluble Dietary Fibre
SPSS	Statistical Package for the Social Sciences
TDF	Total Dietary Fibre
TPA	Texture Profile Analysis
TS	Total Starch
w/v	Weight per Volume
w/w	Weight per Weight
WHC	Water Holding Capacity
WHO	World Health Organization
y	Year
μL	Microlitre

1. INTRODUCTION

1.1 Overview

In recent years, consumers are becoming more health conscious of the foods they consume. Consumer trends in the meat industry, in particular are shifting towards lowering the fat, cholesterol and salt within their meat products, while maintaining high-levels of protein. In the case of fat, a reduction in comminuted meats may lead to undesirable texture, reduced production yields, soft mushy interiors, excessive purge, and changes to sensory qualities. Non-meat substitutes especially from legumes such as soy, bean, pea, lentil and chickpea have been already used as binders and extenders in comminuted meat products because of their high nutritional value and acceptable functional properties and in some cases reduce product cost. However legumes are also rich in starch and insoluble fibre which can lead to improved physico-chemical and technical properties such as achieving high cooked product yields, and water and fat binding capacities (Chang & Carpenter, 1997; Pietrasik & Janz, 2010).

Chickpea *(Cicer arietinum* L.) is an economically important and highly nutritious seed, ranked as the world's second largest pulse crop based on total global production (Saskatchewan Pulse Growers, 2013). According to Canadian Grain Commission (2012) and the Saskatchewan Pulse Growers (2013), Canada is the worlds' fourth largest exporter of chickpea. Saskatchewan and Alberta are the main regions growing Kabuli chickpea, with Saskatchewan claiming ~83% of Canada's chickpea crop. Moreover, chickpea is rich in protein containing essential amino acids, fibre, carbohydrates, vitamins and minerals; especially, B vitamins, folate, manganese, iron, and copper which are all required for human health and well-being.

Micronization is a short time high-intensity infrared treatment commonly used on grain legumes, including chickpea, along with tempering to reduce cooking times for a wide variety of applications. Tempering or soaking of seeds in water prior to micronization causing swelling or increase in seed size/volume due to water absorption. Tempering is often used as a pre-treatment prior to micronization in order to control moisture levels within the seed and to avoid roasting. Consequently upon milling, seeds are more easily broken up (Arntfield et al., 1997; Fasina et al., 2001). Moisture-temperature interactions during seed processing may lead to unique physico-chemical changes in the resulting flours, specifically the starches and protein, as water molecules are heated from within (Cenkowski & Sosulski, 1996, Fasina et al., 2001).

The overall goal of this research is to investigate the effect of seed tempering moisture and micronization temperature on the physicochemical properties of chickpea flour and its subsequent performance as a binder in a model low-fat pork bologna product. This work was divided into three studies. In the first study, the effect of seed surface micronization temperature (115, 130, 150 and 165ºC) and seed tempering moisture (i.e., untempered (7% moisture), or tempered to 15 or 22% moisture) on the physical, chemical and functional properties of chickpea flour was investigated. Its potential as a binder within low-fat comminuted meat products was also investigated in study two, as an alternative to wheat flour, as a means to hold water and fat to alleviate quality losses (i.e., texture and sensory) associated with reduced fat formulations. Bologna serves as an excellent model for a comminuted meat product, as it has been well characterized by many groups (Yang et al., 2001; Pietrasik & Janz, 2010; Sanjeewa et al., 2010). Selected seed tempering moisture and micronization temperature on the performance of chickpea flour were chosen from study one for incorporation into the model low-fat pork bologna product, based on their functional attributes. In study two, both the texture and sensory properties of the bologna were explored. In study three, a consumer panel was used to better understand consumer purchasing behavior as it relates to purchasing comminuted meat products which include pulse-based binders.

1.2 Hypotheses

The following hypotheses were tested in support of the overall project goal:

1. Increased levels of seed moisture due to tempering will result in improved flour functionality.
2. Increased micronization temperatures of seed will have a positive effect on flour functionality up to a critical temperature; afterwards a detrimental effect will occur.
3. Use of tempered and micronized chickpea seed prior milled to yield flour will improve the texture and sensory properties of the low-fat pork bologna product over un-treated chickpea flour from non-micronized seed.

1.3 Objectives

1. To investigate the effects of seed tempering moisture and micronization temperature on the physical, chemical and functional properties of chickpea flour.
2. To incorporate selected chickpea flours from tempered/micronized seed within a low-fat bologna model system as a binder; and investigate their effects on the textural and sensory attributes of the final product

2. LITERATURE REVIEW

2.1 Chickpea

2.1.1 Origin and production

Chickpea *(Cicer arietinum* L.) is an ancient crop that belong to the family *Leguminosae*, named for their ability to fix nitrogen from the atmosphere. Chickpea was thought to be first introduced around 7000 B.C. in southeastern Turkey and was prevalent in semi-arid zones of India and Middle Eastern countries (Goodwin, 2003). Currently, chickpea is known as an economically important and highly nutritious crop, representing approximately 15% of the total global legume market, and ranks second in terms of production worldwide next to dry bean (Goodwin, 2003; Tar'an, 2013; Saskatchewan Pulse Growers, 2013). The world chickpea production area is ~11.3 million ha, with production of 9.6 million metric tonnes and an average yield of 849 kg/ha during 2006 - 2009. The largest chickpea-producing country is India, accounting for 66% of global chickpea production, which produced 6.38 million metric tonnes of chickpea during 2006 - 2009 (Jukanti et al., 2012). Canada is the worlds' fourth largest exporter of chickpea, exporting approximately 70,100 tonnes in 2007 to primarily Jordan (15%), United States (9%) and Italy (8%) (Agriculture and Agri-Food Canada, 2008). According to the Canadian Grain Commission (2012) and Saskatchewan Pulse Growers (2013), Saskatchewan produced ~83% of chickpea crop grown in Canada.

There are two different bio-types of cultivated chickpea, Kabuli and Desi. The seed of Desi-type are small (120 - 320 g/1000 seeds) and irregular in shape, with a thick, hard and coloured testa (seed coat) varying in colour from green to purple, brown or black. Kabuli-type chickpea, also known as garbanzo bean, are large (260 - 600 g/1000 seeds), ram-head shape, with cream to white-coloured thin testa and smooth seed surface. Kabuli chickpea plants are taller, lower yielding, later maturing, and less resistant to disease and insect damage than Desi chickpea (Foundation of Saskatchewan Agriculture, 2013; Tar'an, 2013). The Desi-type chickpea are mostly grown in Asia and Africa accounting for about 80 – 85% of the world's chickpea production, with West Asia, North Africa, Europe, and North America responsible for the remainder of the Kabuli type (Jukanti et al., 2012).

2.1.2 Composition

Legumes, including chickpea, are increasingly being used in healthy diets in order to promote general well-being and to reduce the risk of disease. Agriculture and Agri-Food Canada (2007) recognizes that chickpea is an important source of protein containing essential amino acids, fibre, carbohydrates, vitamins and minerals; especially, B vitamins, folate, manganese, iron, and copper. Messina (1999), Finley (2007), Bazzano (2008) and Pittaway et al. (2008) reported that fibre from legumes, including chickpea, helps to lower the risk of coronary and cardiovascular disease by reducing blood cholesterol levels and help people with diabetes by lowering blood glucose levels. Dietary fibre from chickpea can be beneficial to colon health and has been associated with reducing the risk of colon cancer (Murillo et al., 2004). In addition, flour made from chickpea is considered gluten-free, a nutritious option for people with celiac disease, and is commonly used in vegetarian diets as a good source of iron and protein (Agriculture and Agri-Food Canada, 2007).

Different environmental factors, such as location, soil-type, level of irrigation and use of fertilizers may have an effect on the compositional breakdown of chickpea (Sanjeewa, 2008). The proximate and mineral composition of Canadian Kabuli- and Desi-type chickpea as determined by the Canadian Grain Commission (2004) is given in Table 2.1. Overall, chickpea are high in protein (~23-24%), carbohydrate (45–50%; with starch representing ~84% of this fraction), fat (~5-6%), ash (~3%) and moisture (~8%) (Hung et al., 1993; Rincón et al., 1998; Alajaji & El-Adawy, 2006). The fiber content of Desi chickpea is slightly higher (ADF 13.1% and NDF 12.8%) as compared with that of Kabuli (ADF 3.7% and NDF 5.0%), suggesting that Kabuli chickpea have higher digestibility than Desi-type. Acid Detergent Fiber (ADF) refers to insoluble fiber that cannot be broken down by the gastrointestinal enzymes, and consists of primarily cellulose and lignin from the plant’s cell wall (Schroeder, 1994). Neutral Detergent Fiber (NDF) is a measure comprised of ADF combined with hemicelluloses and other insoluble fibers (Schroeder, 1994). Chickpea is rich in minerals such as potassium, calcium, phosphorous, copper, iron, magnesium, manganese and zinc.

Table 2.1 Proximate and mineral composition of chickpea produced in Canada. Modified from the Canadian Grain Commission (2004)

	Kabuli type		Desi-type	
	Mean	Range	Mean	Range
Composition (g/100g dry matter)				
Protein	24.4	17.9-30.8	23.0	20.3-27.5
Starch	41.1	38.2-43.9	36.4	33.1-40.4
Amylose (% of total starch)	26.2	24.2-29.2	23.8	20.5-25.9
ADF	3.7	3.0-5.7	13.1	12.7-13.5
NDF	5.0	4.2-7.7	12.8	10.1-13.6
Fat	5.9	5.5-6.9	5.4	4.4-5.9
Ash	3.2	2.9-3.8	3.2	2.7-3.5
Minerals (mg/100 g dry matter)				
Calcium (Ca^{2+})	106.6	80.5-144.3	161.7	115.0-226.5
Copper (Cu^{2+})	1.0	0.7-1.4	1.0	0.5-1.4
Iron (Fe^{2+})	5.5	4.3-7.6	5.9	4.6-7.0
Potassium (K^{+})	1127.2	816.1-1580.1	1215.7	1027.6-1479.1
Magnesium (Mg^{2+})	177.8	152.9-212.8	169.1	143.7-188.6
Manganese (Mn^{2+})	3.9	2.3-4.8	3.4	2.8-4.1
Phosphorus (P^{3-})	505.1	294.1-828.8	377.3	276.2-518.6
Zinc (Zn^{2+})	4.4	3.6-5.6	3.6	2.8-5.1

Notations, ADF and NDF refer to acid and neutral detergent fibre, respectively.

(a) Proteins

In terms of the protein content, like other legumes, the major proteins in chickpea are comprised of the salt-soluble globulins and the water-soluble albumins. The latter primarily consists of enzymatic proteins, enzyme inhibitors and lectins. In contrast, globulins are comprised of both a hexameric legumin protein (11 S; S- Svedberg unit; molecular mass of ~350-400 kDa) and a trimeric vicilin protein (7S; molecular mass of ~150 kDa). The legumin protein is comprised of 6 subunits (each ~60 kDa) held together by hydrophobic interactions and non-covalent bonding, with each subunit consisting of both an acidic (~20 kDa) and basic (~40 kDa) chain joined together by disulphide bridging (Tzitzikas et al., 2006). The vicilin protein is a trimer of ~50 kDa subunits held together by non-covalent bonding and hydrophobic interactions. Other minor proteins include prolamins (soluble in aqueous alcohol) and glutelins (soluble in diulte acid or alkali detergents) (Tzitzikas et al., 2006; Emami & Tabil, 2002). Boye et al. (2010) reported protein profiles of albumins (~8.4-12.3%), globulins (54.4-60.3%), prolamins (3.1-6.9%) and glutelin (19.4-24.4%). Proteins play an important role in terms of offering essential amino acids, along with their functional attributes such as water- and lipid binding properties, emulsion capacity. These properties refer to the ability of the the protein to absorb water or lipid to the protein's surface or within its interior (Damodaran, 2008).

(b) Starch

Starch is the most abundant carbohydrate in the legume seed including chickpea. Legume starches are digested slowly, have a low glycemic index and are fermented in the large intestine to produce short-chain fatty acids that are beneficial for colon health (Hughes et al., 2009; Miao et al., 2009). Starch is accumulated in the form of starch granules composed of two polymers: a linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin. Chickpea seed has amylose contents ranging between 27-34% of the total starch (Singh, 1985; Singh et al., 2004), with the remainder being amylopectin. Amylose is a linear polysaccharide chain consisting of (1$\rightarrow$4)-linked α-D-glucopyranosyl units, with only few branches connected by α-D-(1$\rightarrow$6) linkages (0.3-0.5% of the linkages). An average molecular mass of amylose is about 500 kDa. In contrast, amylopectin is a very large, highly branched molecule, with branch point linkages constituting 4-5% of the total linkages. The molecular mass of amylopectin may be as high as 100,000 kDa (Ryan et al., 2006; BeMiller & Huber, 2008). Starch granules are insoluble; and hydrate only slightly in cold water. As a result, they can be dispersed in water to produce low viscosity slurries. Gelatinization is the disruption of molecule order within the starch granules. When starch is heated in water, the hydrogen bonds holding the starch together weaken, allowing water to penetrate the granule, causing them to swell. Amylose then migrates out of the granule. When the starch granules rupture, starch polymer alignment occurs. This phenomenum results in a decrease of paste viscosity (BeMiller & Huber, 2008). The thickening properties of starch occurs when the slurry is cooked by heat to a specific temperature, known as the gelatinization temperature. Beyond the gelatinization temperature, continued heating of starch granules in excess water results in swelling of more granules, hydration of more water, and the disruption of molecular order within the granules leading to an irreversible starch paste (Kaur et al., 2007). Moreover, upon cooling the amylose molecules aggregate, leading to gel formation (BeMiller & Huber, 2008). The degree to which starch is gelatinized within a food system is generally dependent upon both the amount of water present and the extent of the heat treatment. The gelatinization property of starch plays a variety of roles in food production used to produce desired texture qualities and water-binding capabilities (BeMiller & Huber, 2008).

(c) Lipids

Food lipids are generally refered to as fats (solid) or oils (liquid) indicating their physical state at ambient temperatures. Moreover, they are also classified as nonpolar and polar lipids to indicate differences in their solubility and functional properties. Polar lipids contain a hydrophilic group that has a high affinity for water; whereas a lipophilic group has a high affinity for oil (McClements & Decker, 2008). The neutral lipids are the predominant class of lipids in most of the legume seeds including chickpea. The triglycerides are the major components of neutral lipids (nonpolar) whereas, lecithin is the major polar lipid component (Singh, 1985). The majority of lipids in chickpea are polyunsaturated fatty acids, especially linoleic acid, an essential fatty acid. The high level of unsaturated fatty acids may influence the functional properties including storage stability of processed flours obtained from chickpea (Sosulski & Gadan, 1988; Attia et al., 1996). In addition, lipids play an essential role in food quality by determining texture, appearance, and flavour of food products affecting consumer acceptability (McClements & Decker, 2008).

(d) Fibre and ash

Non-starch polysaccharides are considered dietary fibre as they are indigestible in the human small intestine. These are found mainly in the plant cell wall (BeMiller & Huber, 2008). Dietary fibre plays an important role in decreasing the risk of diseases such as diabetes and cardiovascular diseases (Eastwood & Kritchevsky, 2005) and consists of two types: soluble and insoluble fibre. Soluble fibre is fermented in the colon and gets converted into gases and physiologically active by-products; while insoluble fibre does not break down since it is metabolically inert and absorbs water as it passes through the digestive system to ease excretion (Brownlee, 2011; United States Department of Agriculture, 2012). According to the United States Department of Agriculture (2012), gastric emptying of ingested foods into the small intestine is delayed by soluble fibers. This causes a sensation of fullness in the stomach that may contribute to weight control. Moreover, the delayed gastric emptying may also reduce postprandial blood glucose concentration and have a beneficial effect on insulin sensitivity. Blood cholesterol concentrations can become reduced due to the soluble fibres which interfere with the absorption of dietary fat and cholesterol and also with the enterohepatic recirculation of cholesterol and bile acids (United States Department of Agriculture, 2012). De Almeida Costa et al. (2006) reported that raw chickpea contained ~13% of insoluble fibre, whereas soluble dietary fibre was not detected. Ramula & Rao (1997) found that dehulled chickpea seeds contained 14.43-16.15%, 11.78-13.68%, 2.39-2.73% total dietary fibre, insoluble dietary fibre, and soluble dietary fibre, respectively. Moreover, chickpea contains about 3-4% ash and is also a good source of minerals such as phosphorus, calcium, magnesium, iron and zinc (Alajaji & El-Adawy, 2006; Iqbal et al., 2006).

(e) Anti-nutritional factors

Certain biologically active substances are commonly considered as anti-nutritional components. They can be found in most food legumes including chickpea (Singh, 1988). One factor limiting the nutritional quality of chickpea is the content of anti-nutrients such as trypsin inhibitors, phytic acid, polyphenols and tannins (Singh & Jambunathan, 1981; Rincón et al., 1998; Khattak et al, 2007). Trypsin inhibitor is a protease which binds and inhibits the enzyme trypsin. This causes inhibition of protein digestibility leading to reduced amino acid availability (Der, 2010). Therefore, digestibility of the sulphur-containing amino acids, limited in majority of the grain legumes, might be impeded. The inhibition of protein digestibility can be prohibited by hydrothermal treatments (Rincón et al., 1998; Champ, 2002). In case of phytic acid, Khattak et al. (2007) found that it can bind to minerals and create a phytate-mineral-protein complex. Consequently, minerals become unavailable for metabolism. In addition, phenolic compounds or their oxidized products can form complexes with essential amino acids, enzymes and other proteins. Subsequently, it lowers their nutritional value and protein digestibility.

2.1.3 Lipoxygenase activity

Lipoxygenase is an iron-containing dioxygenase that catalyses the oxidation of polyunsaturated fatty acids containing a *cis*,*cis*-1,4-petadiene system to produce conjugated unsaturated fatty acid hydroperoxides (Sanz et al., 1994; Robinson et al., 1995; Loiseau et al., 2001). Lipoxygenases are commonly found in plants and animal tissues. Lipoxygenase plays an important role in the genesis of volatile and aroma compounds in plants including free radical production. Free radicals affect other compounds, for instance, vitamin, phenolics and proteins (Robinson et al., 1995). Emami & Tabil (2002) stated that lipoxygenase-produced flavour and aroma compounds are desirable in many foods but the enzyme may also responsible for off-flavours and decreases its nutritional value.

Legume seeds are known to have high level of lipoxygenase activity, particularly in soybean. According to Loiseau et al. (2001), soybean protein consists of 1-2% lipoxygenase enzyme. Sessa (1979) and Iassonova et al. (2009) found that specific volatile aldehydes and alcohols (*e.g.* 2-*n*-pentylfuran and 3-*cis*-hexenal) oxidized from linoleic and linolenic acids contribute to the green-beany flavour of soybean. During seed storage, alkaline extraction, or storage of protein isolate, lipid oxidation can occur which can negatively affect the quality of legumes. Emami & Tabil (2002) found that even in low fat-containing legumes, enzyme lipoxygenase can cause off-flavour during storage. Lipoxygenase activity can be inhibited or inactivated by the addition of heat, acid, alcohol, or antioxidants (Sessa, 1979; Attia et al., 1996; Iassonova et al., 2009).

2.2 Properties of chickpea

2.2.1 Colour and functional properties of chickpea

The suitability of chickpea flour in specific food applications can be influenced by both its colour and functionality. Hunter colour values of chickpea based on L*, a*, b* dimensions from CIE Lab system have been used previously for measuring chickpea flour colour. Kaur & Singh (2005) and Sanjeewa (2010) found that Kabuli chickpea flour was lighter (higher L* value) and had more yellow (higher b* value) colour than Desi-type flour.

The water holding and oil absorption capacities are important attributes of flours, especially in meat applications. Water holding capacity (WHC) is the amount of water (g) per gram of flour it can hold. Kaur & Singh (2005) reported that Indian Desi chickpea flours had

WHC values that ranged from 1.33 – 1.47 g/g. In contrast, Sanjeewa (2010) found that the WHC of Canadian Kabuli chickpea flours ranged from 0.76 – 0.81 g/g. The oil absorption capacity (OAC) describes the amount of oil (g) per gram of flour it can hold. Desi chickpea flours were reported to have OAC values between 1.05 - 1.17 g/g by Kaur & Singh (2005), whereas Sanjeewa (2010) reported Kabuli-type to have OAC values between 0.80 - 0.87 g/g.

2.2.2 Pasting properties of chickpea flour

The changes seen in the starch due to continuing heating after gelatinization has taken place called pasting. Gelatinization is the disruption of molecule within starch granules when starch is heated in water (BeMiller & Huber, 2008). Moreover, pasting refers to the characteristics of starch granule which are swelling, leaching of starch components, and changing in viscosity when shear force is applied (Tiwari & Singh, 2012). Pasting behavior of starches can be measured under controlled heating and constant shearing conditions by using a Rapid Visco Analyzer (RVA) (Tiwari et al., 2011). Parameters obtained from the RVA include pasting temperature, peak viscosity, breakdown viscosity, and final viscosity. During the initial stage of increasing temperature, starch granules begin to swell to several times their original size as they absorb water. The temperature at which viscosity starts to increase is known as the pasting temperature, the minimum temperature required to cook the starch. As the temperature increases, the viscosity also increases rapidly until it reaches a maximum value called peak viscosity. The peak viscosity corresponds to the point that most of granules are swollen. Therefore, it provides an idea of water holding capacity of the starch. After reaching this maximum value, the starch granules rupture under continuous stirring, leading to a decrease in viscosity. This is known as breakdown and relates to the starch paste stability. As the paste is subsequently cooled, the amylose molecules aggregate leading to gel formation resulting in a second increase in viscosity. The starch retrogradation tendency or syneresis of flours upon cooling of cooked flour pastes can be determined by the viscosity increase on cooling which is known as setback. Final viscosity is the viscosity of the starch paste upon cooling to a temperature of 50^{o}C, and indicates the ability of material to form a viscous paste or gel after cooking and cooling (Kaur & Singh, 2005; Sanjeewa, 2010; Singh et al., 2010; Tiwari et al., 2011). According to Kaur & Singh (2005), pasting temperature of flours from different chickpea cultivars ranged from 73.1 to 75.2^{o}C. Peak viscosity of different chickpea flours varied from 12.3

to 180.3 rapid visco units (RVU). Final viscosity and setback of chickpea flours ranged from 126.3 to 225.3 and 19.8 to 62.8 RVU respectively. Breakdown values of flours from various chickpea cultivars ranged from 5.9 to 22.4 RVU. Singh et al. (2010) reported pasting temperature of flours from different chickpea cultivars ranged from 75.0 to 87.1^{o}C. Peak viscosity of different chickpea flours varied from 47 to 71 RVU. Breakdown value of chickpea flours ranged between 2.7 and 10.25 RVU. Final viscosity and setback of different chickpea flours ranged from 47.8 to 80.75 RVU and 7.0 to 15.4 RVU, respectively. Flour from Kabuli chickpea cultivar had low pasting temperature, highest peak viscosity, final viscosity and setback than Desi-type chickpea (Kaur & Singh, 2005; Singh et al., 2010).

2.3 Micronization

Micronization is the applicaton of short time high-intensity infrared heat which infiltrates into products by transmitance as waves. This dry-heating process involves the exposure of a material to electromagnetic radiation on a vibrating bed at wavelengths of 1.8 - 3.4 µm (Zheng et al., 1998; Fasina et al., 2001; Ryland et al., 2010; Enami et al., 2011). Micronization has been used for various purposes, such as coatings, ink, paper board and textiles. For plant materials, it is a continuous dry heating process to precook grains before use in human food and animal feed. Infrared heating has been applied to various seeds including barley (Enami et al., 2011), lentils (Cenkowski & Sosulski, 1996; Scanlon et al., 2005; Ryland et al., 2010), peas (Arntfield et al., 2004), cowpea (Mwangwela et. al., 2007), beans (Belllido et al., 2006), chickpea (Der, 2010), cereals (wheat, barley, rye, triticale, millet, and wildrice) and legumes (green pea, yellow, pea, lentil, black bean, kidney bean, and pinto bean) (Zheng et al., 1998) to achieve various objectives. The micronization of legumes seed leads to protein denaturation, structural changes and variation in starch properties (Ma et al., 2011). Fasina et al. (2001) stated that the penetration of infrared waves into biological material causes the water molecules to vibrate, leading to rapid internal heating, increased stress of water vapour inside the material and rapid water evaporation. Prolonged exposure of a biological material to infrared heat results in the swelling and eventual fracturing of the material.

Moreover, micronizing conditions can affect the activity of anti-nutrients, reduce microbial activity and inactivate enzymes so as to increase food safety and shelf stability (Der, 2010). A reduction of trypsin inhibitor activity in green peas (28%), black bean (52%), and lentils (31%) was observed by Fasina et al. (2001) when micronization to 140°C was applied to these untempered legume seeds. Khattab and Arntfield (2009) investigated the effect of different heat treatments, including micronization, on the anti-nutrients in cowpea, pea, and kidney bean. The levels of anti-nutritional factors such as tannins, phytic acid, trypin inhibitor, oligosaccharides in legumes seed tempered to 24% seed moisture following micronization to 90°C was decreased. In addition, Der (2010) found that micronization of lentils seeds at 15% seed moisture to reach 135°C surface temperature had reduced lipoxygenase activity 100-fold, from approximately 2,000,000 to 20,000 enzyme units/gram protein. However, Khattab & Arntfield (2009) found that boiling, roasting, autoclaving, or microwaving gave the higher ability to decrease anti-nutritional factors than micronization.

Furthermore, in terms of food spoilage issues, micronization is a potential means to increase food safety. Inactivation of bacteria, spores, yeast and mold in liquid and solid foods can be achieved using micronization by causing damage to DNA, RNA, ribosome, cell envelope and protein in microbial cells (Hamanaka et al., 2006). Nevertheless, it is essential to study the effect of variation in micronizing parameters because of the specific inactivation necessities of different organisms. When infrared heating is utilized to inactivate microorganisms, the effect of inactivation relied on micronizing power, temperature of food sample, peak wavelength, sample depth, type of microorganism, type of food materials, and moisture content (Krishnamurthy et al., 2008).

2.3.1 Effect of tempering along with micronization on legumes

Soaking or tempering is a step used in the traditional preparation of many legumes which shortens the cooking time by ensuring penetration of water into the seed (Arntfield et al., 1997). The process leads to an increase in the degree of starch gelatinization and a decrease in protein solubility due to denaturation of proteins upon cooking (Zheng et al., 1998; Scanlon et al., 2005; Bellido et al., 2006). Tempering is often used as a pre-treatment prior to micronization in order to control moisture levels within the seed. Micronization with appropriate tempering conditions has been used as a pre-cooking treatment to decrease cooking times through the precooking of starch, denaturation of protein, and improving hydration rate of the seeds; however, it has many effects on the physicochemical characteristics (Cenkowski & Sosulski, 1996; Scanlon et al., 2005; Bellido et al., 2006; Mwangwela et al., 2007). Studies have reported the beneficial effect of seed tempering prior to micronization on increased gelatinization. Arntfield et al. (1997) and Bellido et al. (2006) found that high seed moisture levels in lentils, navy and black beans during the gelatinization process is required since moisture is essential for the starch granules to swell and the removal of amylose, while proteins become more susceptible to denaturation and aggregation during micronization. The micronization temperature and initial seed moisture content have to be controlled in order to avoid browning, burnt seed coat, Maillard-type reactions, and excessive starch gelatinization, and untimely protein denaturation (Cenkowski & Sosulski, 1996; Scanlon et al., 2005).

Although tempering with water alone has been shown to reduce cook times of legumes, other tempering solutions may be more effective such as, dilute salt, acidulates, and/or alkali solutions (Bellido et al., 2006). Arntfield et al. (1997) found that in an alkaline environment, beans were softer and cooking times reduced, presumably due to replacement of divalent cations with monovalent sodium. Carbonates were reported to be most effective in this respect. The use of polyphosphates and ethylene diamine tetra acetic acid (EDTA) to chelate divalent cations has also been shown to reduce cooking times. The effectiveness of these additives is dependent on the amount of water available during the tempering pretreatment. Bellido et al. (2006) reported that the mixture of sodium bicarbonate, sodium carbonate and dibasic sodium phosphate was more effective than the mixture of citric and ascorbic acid or disodium EDTA or water alone in reducing the hardness of micronized black beans seed with a seed tempering pre-treatment of 26% moisture seed content, while the water and salt mixture were equally effective in reducing the hardness of navy beans and the firmness of both navy and black beans when tempered to 28% and 26% moisture respectively. Bellido et al. (2006) stated that chelating agents such as EDTA facilitate cell wall separation during cooking through ion exchange and chelation mechanisms between monovalent cations (Na^+, K^+) in solution and divalent cations (Ca^{+2}, Mg^{+2}) in the middle lamella which help to soften the texture of beans. Furthermore, salt solutions containing high ionic strength anions are thought to accelerate protein denaturation and thus shorten the cooking times of beans.

2.3.2 Effect of micronization on the physicochemical, functional and pasting properties of legume flour

Legumes contain high amounts of starch and protein that gelatinize and denature, respectively, in the presence of excess moisture and heat (Zheng et al., 1998; Scanlon et al., 2005). Arntfield et al. (1997) reported that a higher moisture level (compared 25%, 29%, and 33% tempering level) of lentil seeds during micronization resulted in an increase in the percent starch gelatinized and was responsible for protein denaturation. If more starch is gelatinized and more proteins are denatured during micronization, less energy and time would be required to complete the gelatinization process during cooking. Therefore, for a set cooking time, lentils, which have more starch gelatinized and protein denatured during micronization, produced a softer product. The physical properties of the seeds are changed due to venting of steam in weak spots, such as intercellular vertices resulting in enhanced porosity. The micronized seed porosity increases because of the rise of tempering moisture. The increased porosity plays an important role on cooking time through stiffness reduction and enhancement of moisture transport. If the moisture movement through pores compared to the solid matrix is faster, the changes in the grain legume associated with cooking are accelerated as a result of changes in starch and proteins by micronization. Therefore, the water uptake will be improved, causing the increased softness at a given cooking time (Scanlon et al., 2005). Moreover, 140°C micronization of legume (kidney beans, green peas, black bean, lentils, and pinto beans) seeds was found to have the low (<10%) amount of gelatinized starch since initial moisture contents were less than 10% (Fasina et al., 2001).

Water holding and oil absorption capacities are considered important properties of legume flour when using it as a binder in comminuted meat products. Micronizing cowpea seed up to 170°C did not change OAC of cowpea flour compared to non-micronized and micronized to 130°C whereas WHC of cowpea flour increased as increasing micronization temperature (Mwangwela et al, 2007). Fasina et al. (2001) reported that legume flour (kidney beans, green peas, black bean, lentils, and pinto beans) from seeds micronized at 140°C absorbed more water than those from non-micronized seed. The increased WHC might be due to protein denaturation and starch gelatinization during micronization (Fasina et al., 2001; Mwangwela et al., 2007).

Mwangwela et al. (2007) found that tracking starch granule swelling and stability of the native and hydrothermally treated legume flours can be accomplished through the use of pasting curves. The characteristic cold swelling peak anticipated in pregelatinized starch was not detected in the flour from micronized (130 and 170ºC) cowpea seeds. Increased micronization temperature caused pasting temperatures to increase. Granule modification likely enhanced pasting temperature of hydrothermally treated starch. Embedding of some starch granules in the denatured protein matrix within parenchyma cells could restrict water access into the granules and other competing hydrophilic molecules such as proteins. Moreover, increased micronization temperature lead to enhanced pasting viscosity (Mwangwela et al., 2007).

2.3.3 Effect of micronization on sensory characteristics

The sensory properties of products containing legume flours are of upmost importance since they can contribute to off-colours and flavours in the final product. The effects of micronizing conditions on legume colour have been investigated. Micronization (130°C) increased the darkness, redness, and yellowness of whole seed of green lentil (Der, 2010). Mwangwela et al. (2007) reported that cowpea flour became darker, redder, and more yellow with an increasing micronized temperature (non-micronized, micronized to 130 and 170°C) of seed. The browning of cowpea and lentil flour with micronization of seed was possibly due to Maillard reactions, since they contain reducing sugars and high protein contents (Mwangwela et al, 2007; Der, 2010). As tempering level increased (25, 29, 33%), there was a significant decrease in the lightness of the lentil seeds (Arntfield et al., 1997). Micronizing non-dehulled lentil seed (33% seed moisture) to 138 and 170°C provided a less bright, more red, and slightly lower yellow colour compared to non-micronized (Arntfield et al, 2001). Similar results found in Emami et al. (2011) in barley seed tempered to 42% initial seed moisture following by micronization to reach 100°C.

For micronized seeds, development of aroma is another quality consideration. Cowpea seeds tempered to 41% moisture following 153°C micronization had higher roasted aroma and flavour compared to non-micronized samples which might due to the flavour compounds such as pyrazines from Maillard reaction during micronization (Kayitesi et al., 2012). The reduction of firmness, mealiness, and coarseness was found in this study. Kayitesi et al. (2012) explained that cracking of seed coat, cotyledon and parenchyma cell wall was the result of micronization. In addition, bulk density of micronized seeds decreased. The micronization also affect they physical structure resulting in softer texture in cooked chickpea. According to Kouzeh-Kanani et al.

(1982), micronization helped maintain the freshness of soybean flour for 1 year whereas non-micronized samples resulted in rancidity development.

2.4 Meat applications for legume flours

2.4.1 Low-fat meat products

Fat is an excellent source of energy and essential fatty acids, and carrier of fat-soluble vitamins. In processed meats, fat is a major determinant of the sensory characteristics of products due to its contribution to both flavour and texture. Fat also plays an important role in stabilizing meat emulsions, reducing cooking loss, improving water holding capacity and providing juiciness and hardness as fats have considerable effects on the binding, rheological and structural properties of the meat product (Pietrasik & Duda, 2000; Mahmoud & Badr, 2011). According to Pietrasik & Duda (2000), and Mahmoud & Badr (2011), fat is one of the factors influencing the stability of meat emulsions. If the fat particles are too large, a stable emulsion cannot be formed which lead to low water holding capacity causing an increase in cook loss and gives rise to a watery product. On the other hand, if the fat is chopped too much the surface area may be too large to make a stable product. Therefore, it is important to reduce the fat (by chopping in meat processing) to a size small enough allowing the extracted protein to coat or entrap the fat. However, the World Health Organization (WHO) has drawn up the following nutritional recommendations that fat should provide between 15% and 30% of the calories in the diet, saturated fat should not provide more than 10% of these calories and cholesterol intake should be limited to 300 mg/day (Serdaroğlu & Değirmencioğlu, 2004). This resulted in an increased demand for fat reduced and low fat meat products by both industry and consumers. Moreover, consumption of too much fat (>30%) in the diet has been reported as a health concern to increase risk of coronary heart disease, and associated with several diseases such as obesity and hypertension (Colmenero, 1996; Carrapiso, 2007; Choi et al, 2009).

According to Carrapiso (2007), varying fat content influences emulsion stability, which could modify the interactions among some components involved in emulsion stability such as protein. In a meat emulsion, protein plays an important role as an emulsifier to help form a stable emulsion between fat and water, too much fat will decrease the stability of the emulsion due to binding ability of protein with fat and water (Knipe, 2012). Fat has been replaced in processed low-fat meat products with added water, non-meat proteins, and polysaccharide gums. Direct substitution of fat with water in low fat versions of emulsified or comminuted meat products may lead to less acceptable texture, reduced production yields, soft mushy interiors, excessive purge, and changes in sensory qualities after cooking or reheating (Colmenero, 1996; Pietrasik & Janz, 2010). Different fiber-rich ingredients have recently been used as functional additives to emulsify meat products and as a binder which resulted in the production of more stable low fat meat products with better textural properties. Legumes, particularly soybeans, have been used as a fat replacer due to their content of insoluble fibre which led to high yields with high water and fat binding capacity (Chang & Carpenter, 1997; Pietrasik & Janz, 2010). Therefore, the replacement of fat with legumes as fat substitutes is an attractive approach in low-fat meat products because of its nutrient composition and ability to maintain the functional properties of the meat product.

2.4.2 The physicochemical and functional properties of plant-based products

Cereal and legume flours have been used as binders and extenders in a wide range of meat applications due to their low cost, nutritional value and good functional properties (e.g., water/lipid holding, emulsification). The term binder is used for substances of animal or plant origin, which have a significantly high level of protein that serves for both water and fat binding, whereas extenders are non-meat substances with substantial protein content, primarily plant proteins from legumes such as soy, beans, peas, lentils. These cheaper plant proteins extend the more expensive meat proteins, resulting in acceptable overall protein contents while producing lower cost meat products (cost reduction and volume increase) (Heinz & Hautzinger, 2007). Moreover, increased utilization of non-meat additives also plays an important role on health benefits such as increasing fiber and mineral content of meat products (Heinz & Hautzinger, 2007). According to the 1990 Canadian Meat Inspection Regulations, Canadian standardized meat products such as sausage (ready to eat) including, salami, wieners, frankfurters and bologna are required to contain a minimum protein content derived from meat (9.5%) and total protein (11%). Different non-meat ingredients, which have been used in different meat systems, are summarized in Table 2.2.

Moharram et al. (1987) found the addition of soy, faba bean, chickpea, and white rice flour into beef burgers led to an increase in moisture by 12-34% and carbohydrate content by 7-29% and a decrease in protein (9-24%), fat (3-5%) and ash (1-5%) except in the case of soybean, which caused a noticeable rise in ash content. These results agree with those reported by Dzudie et al. (2002) with addition of common bean flour at levels of 2.5%, 5.0%, 7.5% and 10.0% into beef sausages and by Prinyawiwatkul et al. (1997) with addition of cowpea and peanut flours to chicken nuggets. Minerich et al. (1991) found that increased level of added cooked wild rice flour into low-fat ground patties (0, 15, and 30%) resulted in a decrease in protein (11-24%), fat (11-27%) and ash (9-18%). Brown & Zayas (1990) found that addition of 10, 20, and 30% corn germ protein flour into beef patties led to a decrease in protein content (8-26%). Moreover, the result from Brown & Zayas (1990) showed that extended beef patties (contained corn germ protein flour) had lower cooking losses and higher cooked yields than control patties. This corresponds with Dzudie et al. (2002) and Minerich et al. (1991) where water holding capacity and cooking losses decreased with increasing levels of added non-meat binders. In addition, Serdaroğlu et al. (2005) reported that meatballs extended with legume (including chickpea) flours had higher water holding capacity than meatballs extended with rusk, a dried bread crumb. Due to the higher protein content of chickpea (20.6% protein) compared with the rusk (12.4% protein), Serdaroğlu et al. (2005) explained that meat protein matrix formed in meatballs containing chickpea is much more stable than meatballs extended with rusk, leading to higher water holding capacity.

However, the presence of binders in meat applications may also lead to a negative effect on texture and sensory attrributes of the product. The combined effects of red pigments in muscle (myoglobin and hemoglobin) mixed with other constituents present in meat generates the natural meat colour. That means percentage of myoglobin and hemoglobin in combination with muscle, fat, iron and connective tissue influences the meat colour (Aberle et al., 2001). Unusual meat colour may develop under various situations. However, some of which are not correlated with the normal chemical reactions of pigments but rather other ingredients formed within the product. For instance, Verma et al. (1984b) reported that during the preparation of sausages containing chickpea flour, brown discolouration of the batter was observed at the mixing stage. Similar findings were observed by Prinyawiwatkul et al. (1997) when chicken nuggets were extended with cowpea and peanut flour. They reported that as the amount of cowpea and peanut flour increased, a more intense orange-brown colour was reported; nevertheless, Verma et al. (1984) explained that chickpea flour itself does possess a yellowish tinge, but subjective and objective assessment indicated that the browning was not related to the inherent colour of the flour itself but appeared to be related to the conversion of the haematin pigments present in the meat (mainly myoglobin) to the brown oxidized form. For the texture charecteristics, Sajeewa (2008) found that bologna with Kabuli and Desi flour at 5.0% showed the highest Allo-Kramer (A-K) shear forces among the treatments. The A-K shear values increased as the level of chickpea flour in the formulation increased suggesting a strong gel structure formation in between meat and flour components. The hardness and cohesiveness values from texture profile analyses (TPA) of legume flour formulated (chickpea and pea) were significantly higher than that of the wheat flour containing bologna which might be due to the difference of protein content between the legume flours and wheat flour. These texture results agree with that reported by Der (2010) with addition of lentil flour into beef burgers. In addition, adding legume flour may lead to graininess and foreign flavour of meat products (Sanjeewa, 2008; Der, 2010).

Table 2.2 Flours from various sources used as binders/extenders in different meat products.

Meat System	Plant Ingredient	Reference
Low-fat pork bologna	Chickpea flour	Sanjeewa (2008)
Low-fat duck sausage	Rice flour	Ali et al. (2011)
Beef sausage	Bean flour	Dzudie et al. (2002)
Fresh sausage	Chickpea flour	Verma et al. (1984)
Frankfurters	Tapioca starch	Hughes et al. (1998)
	Oat bran	Chang & Carpenter (1997)
Low-fat meat balls	Legume flour (chickpea, bean, lentil and rusk)	Serdaroğlu et al. (2005)
Low-fat beef burger	Lentil flour	Der (2010)
Buffalo meat burger	Soy, faba bean, chickpea, Bengal gram, black gram and rice flour	Moharram et al. (1987); Modi et al. (2003)
Chicken nuggets	Cowpea and peanut flour	Prinyawiwatkul et al. (1997)
Beef patties	Oat flour	Serdaroğlu (2006)
Groung beef mixture	Wild rice	Minerich et al. (1991)

3. Study 1: The effect of seed tempering and micronization temperature on the physciochemical and functional attributes of chickpea (Kabuli-type) flour

3.1 Abstract

The effect of seed tempering moisture (i.e., untempered, 7% seed moisture) or tempered to 15% or 22% moisture) and surface micronization temperature (115, 130, 150, and 165^{o}C) on the physicochemical and functional properties of kabuli-type chickpea flour were investigated. Both micronization temperature and tempering conditions of chickpea seed were found to influence the colour of chickpea flour where flours became lighter (higher L* value) at lower micronized temperature and lower seed moistures. In contrast, the flours became more yellowish in colour (higher b* value) as the micronization temperature of seed increased. Level of gelatinized starch significantly (p<0.05) increased as the micronization temperatures were raised when chickpea seed was tempered to 22% moisture, whereas gelatinized starch was not detected in flour from seed tempered to 7% and 15% seed moisture. Values for lipoxygenase activity of fresh ground chickpea flour from non-micronized seed was found to be 3.37×10^{5} units/g of protein and 1.98×10^{5} units/g of protein for flour from non-micronized seeds ground and stored for 2 years. Lipoxygenase activity of flour from untempered chickpea seed which was micronized to 115^{o}C was found to be 1.12×10^{5} units/g of protein whereas, no activity was found in any other treatments. The functional properties and water holding capacity (WHC) of chickpea flours increased with increasing levels of seed tempering moisture and surface micronization temperature. Oil absorption capacity (OAC) increased as the level of seed tempering moisture increased, however OAC was found to be independent of micronization temperatures at the 7% (untempered) and 22% seed moisture levels, but not at the 15% seed moisture. The rapid visco analyzer (RVA) data on chickpea flour showed that the pasting temperature of flours increased as the level of seed tempering moisture increased, whereas final viscosity decreased with increasing micronization temperatures when chickpea seed was tempered to 15 and 22% moisture.

3.2 Introduction

Non-meat ingredients such as cereal and legume flours, including chickpea, have been used as binders and extenders in a wide range of meat systems due to their low cost, nutritional value and functional properties (e.g., water/lipid holding capacity). Chickpea represents about 15% of the world pulse market. Its production area is ranked second, and is the third largest pulse crop exported worldwide (Goodwin, 2003; Tar'an, 2012; Saskatchewan Pulse Growers, 2013). Some studies showed the positive impact from the addition of legume flour into meat products such as the effect on nutritional value (Moharram et al., 1987; Minerich et al., 1991; Prinyawiwatkul et al., 1997; Dzudie et al., 2002), lower cooking losses and higher cooked product yields (Brown & Zayas, 1990; Minerich et al., 1991; Dzudie et al., 2002; Serdaroğlu et al., 2005). However, the presence of binders in meat applications may result in an adverse effect on texture as well as on the sensory properties of the final product such as colour, graininess, and the presence of foreign flavour (Verma et al., 1984b; Prinyawiwatkul et al., 1997; Mwangwela et al., 2007; Sanjeewa, 2008; Der, 2010).

Micronization is an infrared treatment commonly used on grain legumes, along with tempering to reduce cooking times, reduction of microbial activity and enzyme inactivation for a wide variety of applications. Tempering or soaking the seeds prior to micronization allows for water to penetrate the seed coat, causing swelling or increases in seed size/volume. Consequently, upon milling seeds are more easily broken up (Arntfield et al., 1997; Fasina et al., 2001). The process also aids in increasing the digestibility and nutritional quality of proteins, especially for those used in feed applications. Moisture-temperature interactions during seed processing may lead to unique physicochemical changes in the flours, specifically the starches and protein, as water molecules are heated from within (Cenkowski et al., 1996; Fasina et al., 2001).

The overall goal of the present study is to investigate the effect of surface micronization temperature (115, 130, 150 and 165°C) and seed tempering moisture condition (i.e., untempered (7%), and tempered to 15 and 22% moisture) on the physical, chemical (proximate composition, colour, gelatinized starch content, lipoxygenese activity) and functional properties (water holding capacity, oil absorption capacity, pasting properties) of chickpea flour, in order to find optimum conditions which would be useful in meat applications.

3.3 Materials and methods

Hull on kabuli chickpea seed used in this study was obtained from InfraReady Products Ltd. (Saskatoon, SK, Canada). They had been split and were yellow cream in colour. Chickpea seeds (with hull) from the same lot was sub-divided into 3 groups: a) untempered seed (7% moisture); b) seed tempered to a 15% moisture level; and c) seed tempered to a 22% moisture level. All groups were micronized to reach four different surface temperatures: 115, 130, 150, and 165°C. Once micronized, seeds were milled to yield a flour, and then analyzed. Non-micronized chickpea flour served as a control. Tempering and micronization of all treatments was performed in duplicate on different dates.

3.3.1 Preparation of the chickpea flours

(a) Tempering

Tempering of the chickpea seeds was performed at InfraReady Products Ltd. (Saskatoon, SK, Canada) according to AACC method 26-95.01 (1999). Approximately 2-3 kg of chickpea seeds with the hull were tempered at ambient temperature for 16 h and 27 h to achieve equilibrium final desired moisture content of 15% and 25%, respectively, in sealed polyethylene bags by adding a pre-determined amount of deionized water; calculated using eq. 1 (AACC 26-95.01, 1999). The final moisture content of chickpea seeds reached 15% and 22%. The moisture content of chickpea seeds before and after tempering were determined using a moisture analyzer.

$$W = \left(\frac{100 - \text{Moisture}_O}{100 - \text{Moisture}_T} - 1\right) \times \text{Chickpea weight} \qquad \text{(eq. 1)}$$

Where, W is the water weight required (g), Moisture_T is the moisture required at tempering (%) and Moisture_O is the moisture content of seeds before tempering (%)

(b) Micronization and milling

Micronization was performed using a laboratory scale micronizer (Model A 156379 –B0, FMC Syntron ® Bulk Handling Equipment, Homer City, PA, USA), composed of a gas-heating element with two sets of three ceramic tiles (Model type R 1603-2 pat, Rinnai, Japan), a Syntron feeder (Model F010, Riley Automatic Ltd., Derby, England) and a Syntron magnetic feeder (Mode BF2 A, FMC Corporation, Homer City, PA, USA) at InfraReady Products Ltd. (Saskatoon, SK, Canada). Approximately 2-3 kg of chickpea seeds (tempered or not) were placed into a hopper which fed onto a moving vibrating bed exposed to overhead infrared lamps positioned 20 cm above. The conveyor belt speed and vibrating bed was adjusted to achieve the required seed surface temperatures of 115, 130, 150 and 165ºC. Surface temperature of the out coming seeds was monitored using a hand-held IR thermometer (Oakton, Vernon Hills, IL, USA). Non-micronized seed served as a control. Micronized seeds were then transferred directly to a roller mill (Apollo Machine and Product Ltd, Custom Built, USA) to form chickpea flakes (3 mm thickness), and then milled at the highest setting using a kitchen mill (Model 91, Blentech, Orem, USA) in order to obtain a size that enables the flour to pass through a 0.5 mm screen. For non-micronized chickpea seeds (control), they were ground using a burr mill (Model No. 60 CM, C.S. Bell Co, USA), and milled twice with the kitchen mill (Model 91, Blentech, Orem, USA) at the highest setting to get non-micronized chickpea flour. Milled flour was collected and vacuum packaged in polyethylene bags and stored at 4ºC.

3.3.2 Physical properties of chickpea seeds

The physical properties of seeds were assessed. The following physical properties were determined in duplicate (Sanjeewa, 2008).

(a) Seed weight (g/seed)

The mean weight of 1000 seeds was examined.

$$\text{Seed weight} = \frac{\text{Weight (g)}}{1000\text{seeds}} \qquad \text{(eq. 2)}$$

(b) Seed volume (mL/seed)

One hundred and fifty seeds were tranfered into a 250-mL cylinder and 100 mL of deionized water was added.

$$\text{Seed volume} = \frac{\text{Total volume (mL)} - 100\,\text{(mL)}}{150} \qquad \text{(eq. 3)}$$

3.3.3 Physical and chemical properties of chickpea flours

Flours from each micronization run were used for measurements of pH, and proximate composition according to AOAC (1990) standard methods. All data represent duplicate samples from each of two replications.

(a) pH

The pH of the flour was determined by the AOAC Method 943.02 (1990) using a pH meter (Model 915, Fisher Scientific, Nepean, ON, USA). One hundred mL of deionized water was added to 10 g of flour at 20ºC and blended using a stomacher (Lab-Blender 400, Model No.BA6021, Seward Laboratory, London, England) for 1 min before measuring the pH.

(b) Moisture (%)

The moisture content of flours was evaluated following the AOAC Method 925.10 (1990). Approximately 2.0 g of flour was weighed into a known weight aluminum pan. Weight of the sample was recorded. The sample in the aluminum pan was dried in an oven at 100–102ºC overnight. The sample pans were then cooled in a desiccator. Pans were re-weighed and the loss was assumed to be moisture.

(c) Crude protein (%)

The nitrogen content was analyzed by Kjeldahl method using the AACC Method 46-11 (1995). Approximately 1.0 g of flour was digested by heating with concentrated H_2SO_4 in a heating/digestion block. After digestion, samples were distilled using a steam distillation unit (Model 320, Buchi Analytical Inc., New Castle, DE) with 30% (w/v) NaOH. Boric acid (4%) was used to trap ammonia from the distillation. The distillate was titrated with 0.2 N HCl, using N-Point indicator as an indicator, to determine the total nitrogen content of the sample. Protein content of the sample was calculated from nitrogen content using the conversion factor of 6.25.

(d) Crude fat (%)

The crude fat content of flours was measured by the AACC Method 30-25.01 (1995). Fat was removed from approximately 2-5 g of flour using petroleum ether as the solvent, using a Gerhardt Soxtherm extractor which was connected to a Multistat system (Model Soxtherm Multi- stat/SX PC, Gerhardt, Königswinter, Germany).

(e) Total ash

The total ash content of the flours was determined according to the AOAC Method 923.03 (1990). Approximately 3-5 g of flour was weighed into a pre-weighed crucible, and was ashed at 550ºC overnight or until a grey mass is formed. The crucible with the ash was cooled in a desiccator and then re-weighed. The weight remaining was determined.

(f) Colour

A Hunterlab MiniScan XE colourimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to determine the colour of chickpea flours based on L^*, a^* and b^* dimensions from the CIE Lab system. Illuminant A and a 10º observer were used. Approximately 10 g of flour was placed within a plastic Petri dish, 5 cm diameter, with the lid on. The colour of flour was measured through the plastic Petri dish lid with flour in direct contact with the lid, two readings per dish and two dishes per sample. The L^* indicates the degree of lightness from dark to light by 0-100, higher the a^* value means more red, and the b^* value represents the intensity of yellow-blue colour with higher positive b^* value signifying more yellow. The instrument was standardized using the black and white tiles. The pink tile was used as a colour check by monitoring its L*, a*, b* value.

(g) Gelatinized starch content

Gelatinized starch content was analyzed using the method of Chiang & Johnson (1977), Der (2010) and Emami et al. (2010). Twenty mg of flour was weighed into a 50-mL centrifuge tube and washed twice with 10 mL of 80% ethanol. Five mL of ethanol was then added, mixed by votexing for 10 s, and then incubated in a 40ºC water bath for 10 min. Afterward, an additional 5 mL of ethanol was added, vortexed for 10 s, and centrifuged at 1,500 × *g* for 10 min at room temperature (21-23ºC) using the rotor SH-3000 (Sorvall Re – 6 plus superspeed centrifuge, Thermo Fisher Scientific, Asheville, NC 28804, USA). After decanting the ethanol, residual solvent was evaporated from the centrifuge pellets by placing them in an oven at 30ºC until the sample was completely dry. Dried starch pellets were dispersed in 5 mL of double-distilled water, followed by the addition of 25 mL of an enzyme amyloglucosidase solution (amyloglucosidase from *Aspergillus niger* having an activity of 60,000 units/g solid was dissolved in 250 mL sodium acetate buffer (pH 4.5) and then filtered through glass microfibre filter paper (Whatman No. GF/A, Whatman International Ltd., Maidstone, England)). The sample was then mixed by vortexing, and incubated at 40ºC in a shaking water bath at 100 rpm for 30 min in order to digest gelatinized starch into glucose. Then, 2 mL of 25% (w/v) trichloroacetic acid was added to stop the reaction. The sample was then centrifuged at 1,500 x *g* for 5 min and supernatant collected. A range of glucose concentrations (0, 25, 50, 75 and 100 μg glucose/0.1 mL) was also prepared in order to generate a standard curve. To 0.1 mL of supernatant or the glucose standard solutions, 3 mL of *o*-toluidine reagent (1.5 g thiourea dissolved in 940 mL glacial acetic acid and 60 mL of *o*-toluidine) was added within the test tube. The tube was then incubated in a water bath at 100ºC for 10 min. After cooling, the absorbance was measured at 630 nm using a spectrophotometer (Shimadzu UV Spectrophotometer, Model UV – 1800, Shimadzu Corporation, Kyoto, Japan) against a reagent blank containing 0.1 mL double-distilled water and 3 mL *o*-toluidine reagent. The starch content was calculated from glucose content on a dry basis as glucose × 0.9.

Gelatinized starch (%) was calculated using the following equation:

$$G = \frac{D - K}{TS} \times 100 \qquad \text{(eq. 4)}$$

where, G (%) = gelatinized starch, D (%) = starch digested by amyloglucosidase, K = correction factor and TS (%) = total starch. K was calculated by weighing out and digesting 5, 10, 15, and 20 mg of native chickpea flour, and determining the glucose released. A curve was developed where the glucose released is linearly related to a corresponding amount of total sugar. K = slope × 100.

(h) Lipoxygenase activity

Lipoxygenase (LOX) activity was determined in chickpea flour using a modified method of McCurdy et al. (1983), Chang & McCurdy (1985), and Der (2010). Fifty mL of phosphate buffer (0.05 M, phosphate buffer, pH 6.9) was added into a 150 mL beaker containing 5 g of chickpea flour, then stirred at 4^{o}C with a magnetic stirrer (VWR standard multi position stirrer, Model 986904, Henry Troemner LLC, USA) at speed 6 for 2 h to extract LOX. The slurry was then centrifuged for 30 min at 11,410 × *g* at 4^{o}C using the rotor F10S – 6 × 500Y (Sorvall Re – 6 plus superspeed centrifuge, Thermo Fisher Scientific, Asheville, NC 28804, USA) and filtered through Whatman #3 paper. A 10 – 100 μL aliquot of this crude extract was transferred to a quartz cuvette containing 3.0 mL of substrate solution (0.0946 mM emulsified linoleic acid substrate), inverted 3 times within 5 s. Absorbance data was then recorded at 10 s intervals for 30 min at an absorbance of 234 nm using a spectrophotometer (Shimadzu UV Spectrophotometer, Model UV – 1800, Shimadzu Corporation, Kyoto, Japan). The slope of the linear region of the curve was approximately proportional to the enzyme concentration, and thus used to calculate the lipoxygenase activity with 1 unit of lipoxygenase activity equivalent to an increase in absorbance of 0.001/min at 234 nm.

The substrate was prepared by mixing 2.8 mL of a linoleic acid solution (1% (w/v) linoleic acid in 95% (v/v) ethanol) and 2.8 mL of a Tween solution (1% (w/v) Tween 20 in 95% (v/v) ethanol), then evaporated to dryness using a rotary evaporator (Buchi Rotavapor, Model R-200, Buchi Labortechnik AG, Switzerland) equipped with a heating bath (Buchi Heating Bath, Model B-490, Buchi Labortechnik AG, Switzerland) at 60^{o}C. Fifty mL of 0.05 M borate buffer (pH 9.0) was added into the evaporation flask followed by 50 mL of 0.05 M phosphate buffer (pH 6.9), then mixed and the pH adjusted to 6.9 using concentrated HCl. The substrate was used within 3 h because if the substrate is left longer than 3 h, the lipoxygenase activity decreases.

3.3.4 Functional properties of chickpea flours

The functional properties of chickpea flours based on dry weight basis were investigated. The following functional properties were determined in duplicate for each of the two micronization runs.

(a) Water holding capacity (WHC)

WHC for chickpea flour was determined according to the AACC Method 56-30.01 (AACC, 1999). There were two steps involved in the method, determination of approximate WHC and determination of true WHC. Five g of chickpea flour was weighed into a pre-weighed 50 mL centrifuge tube. Distilled water was then added in small unmeasured increments and stirred with a glass rod after each addition until the mixture was thoroughly wetted. Then the tube was centrifuged for 10 min at 2,000 × *g* by using the rotor SH-3000 (Sorvall Re – 6 plus superspeed centrifuge, Thermo Fisher Scientific, Asheville, NC 28804, USA). The slight amount of supernatant was discarded before weighing the tube. An approximation of water holding capacity for the sample was calculated using eq. 5.

$$\text{Approximate WHC, g/g} = \frac{(\text{Weight of tube} + \text{Sediment}) - (\text{Weight of tube} + 5.0)}{5} \quad \text{(eq. 5)}$$

For determining the true WHC, weight of chickpea flour was computed using eq. 6.

$$\text{Weight of chickpea flour} = \frac{15}{\text{Appoximate WHC} + 1} \qquad \text{(eq. 6)}$$

where, 15 is the desired total weight of sample and water.

Four centrifuge tubes were prepared with added volumes of water: 1.5 and 0.5 mL more and 1.5 and 0.5 mL less than that calculated (15 – weight of chickpea flour). Then the contents of each tube were mixed with a glass rod for 2 min and centrifuged for 10 min at 2,000 × g. The two adjoining tubes, one with and one without supernatant, represent the range in which WHC value occurs. WHC value was presented as midpoint of these two volumes divided by g of flour weight.

(b) Oil absorption capacity (OAC)

The oil absorption capacity of chickpea flour was determined by using the method of Sanjeewa (2008) which was expressed as grams of corn oil bound per gram of the sample on a dry weight basis. Centrifuge tubes were weighed previous to addition of 1 g of samples. Then 10 mL of commercial corn oil was added. The sample in oil was stirred by using a glass rod for 1 min. After a holding period of 30 min, the tubes were centrifuged for 25 min at 3,000 × *g* by using the rotor SH-3000 (Sorvall Re – 6 plus superspeed centrifuge, Thermo Fisher Scientific, Asheville, NC 28804, USA). After that these tubes were left for 25 min in order to drain any remaining oil before re-weighing. OAC was calculated using eq. 7.

$$\text{OAC, g/g} = \frac{(\text{Weight of tube} + \text{Sediment}) - (\text{Weight of tube} + \text{Weight of sample})}{\text{Weight of sample}} \qquad \text{(eq. 7)}$$

(c) Pasting properties

Pasting properties of each flour was determined using a Rapid Visco Analyzer (RVA) (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) following the AACC Method 76-21.01 (1999). Each sample was run in triplicate for each replication. The flour sample (14% moisture basis) was prepared by mixing with water based on moisture content. A sample was weighed and tranferred into a test canister, based on eq. 8.

$$S = \frac{(100-14)\times 3.5}{100-M} \qquad \text{(eq. 8)}$$

$$W = 25 + (3.5 - S)$$

where, S is the sample weight, M is the moisture content (%) and W is the water volume for a sample.

A plastic paddle was used to jog the flour suspension up and down for 10 times, and then placed into the canister. Standard Profile 2 was used according to AACC Method 76-21 (1999) where the starch-water suspension was equilibrated at 50°C for 1 min, increased to 95°C at 6°C/min, held at 95°C for 1.5 min, cooled from 95°C to 50°C at 6°C/min, and final phase was held at 50°C for 2 min. Values in centipoise (cP)/rapid visco unit (RVU) for peak viscosity, trough, breakdown, final viscosity and setback, as well as peak time (min) and pasting temperature (°C) was obtained from the viscograms using Thermocline software which was connected to the Rapid Visco Analyzer.

3.3.5 Statistical analysis

The mean and standard deviations of data were calculated based on the duplication of sample measurements and two replications for 13 chickpea flour samples (non-micronized, three seed tempering moisture conditions at four different surface temperatures of micronization). Data were analyzed by a one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) software (SPSS 16.0, SPSS, Inc., Chicago, IL). The comparison between individual treatment means was assessed by the least significant difference (LSD) procedure. The significance was declared at $p<0.05$. A two-way ANOVA using the Statistical Package for the Social Sciences (SPSS) software was applied in order to see the interaction between micronization temperatures and seed tempering moisture conditions without considering chickpea flour from non-micronized seed as one of the treatments. The level of significance was set at $p<0.05$. Pearson correlation coefficients (SPSS 16.0, SPSS, Inc., Chicago, IL, USA) were determined among the various parameters tested ($p< 0.05$).

3.4 Results and discussion

Hull-on kabuli chickpea seeds used in this study was obtained from InfraReady Products Ltd. (Saskatoon, SK, Canada). The seed weight and seed volume at 11.66% seed moisture was found to be 44 g/100 seed and 36 mL/100 seed, respectively. The chickpea seed was considered as the large kabuli type (North American Grain Corporation, 2005; Sastry et al., 2007).

3.4.1 Physical and chemical properties of chickpea flours

(a) Proximate composition and pH of chickpea flour

The proximate composition and pH values of chickpea flour after treated with different seed tempering moisture and micronization conditions are displayed in Table 3.1 on a dry weight basis. Chickpea flour from non-micronized seed contained the highest moisture (7.32%) relative to all chickpea flours from untempered seed following micronization (1.50-4.45%) (Table 3.1). For all tempering conditions, moisture levels in the flours decreased as the micronization temperature of seed increased. In the case of tempering (compared at corresponding temperatures), only tempering to 22% initial seed moisture lead to higher (p<0.05) moisture in the final flours; especially when seeds were micronized to 115, 130 and 150ºC since the micronized time and/or temperature was not enough to evaporate the moisture. There was no significant (p<0.05) difference of moisture content for chickpea flour from untempered chickpea seed when micronized to reach 165ºC, and flour from seed tempered to 15% seed moisture and 22% seed moisture before micronization to 165ºC. Flour from untempered seeds showed relatively similar moisture values with treatments from seed tempered to 15% seed moisture at corresponding micronized temperatures which means micronization removed sufficient moisture from 15% tempered seed to end up with the same moisture content of untempered seed.

Table 3.1 Proximate compositions of chickpea flours on a dry weight basis. Data represent the mean ± one standard deviation (n = 2).

Treatment	Moisture (%)	Protein (%)^{ns}	Fat (%)^{ns}	Ash (%)	pH
Untempered					
Non-micronized	7.32 ± 1.56^{bc}	19.97 ± 0.12	7.62 ± 0.14	2.82 ± 0.04^{abc}	5.96 ± 0.07^{d}
Micronized to 115^{o}C	4.45 ± 1.17^{def}	19.92 ± 0.19	7.20 ± 0.70	2.77 ± 0.02^{cd}	6.01 ± 0.06^{cd}
Micronized to 130^{o}C	3.54 ± 0.92^{efg}	19.92 ± 0.05	7.55 ± 0.66	2.77 ± 0.04^{cd}	6.04 ± 0.02^{bcd}
Micronized to 150^{o}C	1.92 ± 0.37^{g}	19.83 ± 0.18	7.20 ± 1.04	2.74 ± 0.05^{d}	6.05 ± 0.04^{abcd}
Micronized to 165^{o}C	1.50 ± 0.01^{g}	20.05 ± 0.35	7.14 ± 0.11	2.75 ± 0.06^{cd}	6.09 ± 0.03^{abc}
Tempered to 15% seed moisture					
Micronized to 115^{o}C	6.27 ± 1.21^{cd}	19.89 ± 0.21	7.32 ± 0.24	2.77 ± 0.11^{cd}	6.08 ± 0.03^{abc}
Micronized to 130^{o}C	5.41 ± 0.42^{cde}	19.87 ± 0.24	7.45 ± 0.30	2.74 ± 0.04^{d}	6.12 ± 0.07^{ab}
Micronized to 150^{o}C	2.53 ± 0.16^{fg}	19.72 ± 0.30	7.70 ± 0.05	2.77 ± 0.04^{cd}	6.14 ± 0.06^{ab}
Micronized to 165^{o}C	1.77 ± 0.89^{g}	19.70 ± 0.40	7.28 ± 0.54	2.76 ± 0.03^{cd}	6.14 ± 0.01^{a}
Tempered to 22% seed moisture					
Micronized to 115^{o}C	11.61 ± 1.71^{a}	20.04 ± 0.20	7.55 ± 0.13	2.80 ± 0.05^{bcd}	6.10 ± 0.01^{abc}
Micronized to 130^{o}C	8.87 ± 0.05^{b}	19.98 ± 0.24	7.62 ± 0.34	2.87 ± 0.04^{a}	6.10 ± 0.01^{abc}
Micronized to 150^{o}C	4.52 ± 0.29^{def}	20.01 ± 0.16	7.23 ± 0.40	2.85 ± 0.04^{ab}	6.07 ± 0.03^{abc}
Micronized to 165^{o}C	2.86 ± 0.31^{fg}	20.02 ± 0.32	7.21 ± 0.12	2.80 ± 0.08^{bcd}	6.06 ± 0.01^{abcd}

$^{a\text{-}g}$ Means within the same column with the same letter are not significantly different (p<0.05)
^{ns} Means within the same column are not significantly different (p<0.05)

There were no significant (p<0.05) effects of seed tempering moisture conditions and micronization temperatures on protein and fat contents in the chickpea flours on a dry weight basis (Table 3.1). Mean values for protein and fat content were 19.92 ± 0.11% and 7.39 ± 0.20%, respectively. The ash content and pH of chickpea flour ranged from 2.74-2.87% and 5.96-6.14, respectively. The protein and ash values from proximate analysis of chickpea flour found in this study are within the range reported for chickpea by others. For instance, Mashus (2010) reported that Kabuli chickpea had 17.8-22.0% protein and 1.9-3.2% ash, whereas the Canadian Grain Commission (2004) reported that Canadian Kabuli chickpea contained 17.9-30.8% protein and 2.9-3.8% ash. The fat content of flours in this study are slighter higher than those found in the literature where Kabuli chickpea had crude fat contents ranging between 4.5-5.7% (Mashus, 2010) and 5.5-6.9% (Canadian Grain Commission, 2004).

Even though fat level was not affected by micronization in the present study, Nielsen (1998) discovered that a higher fat content was reported for micronized compared to non-micronized seed. This could be explained in that bound lipids were released due to the heat treatment leading to higher effeciencies of fat extraction during the reflux of solvent on the Goldfish equipment. These proximate composition values can vary considerably with variety, growth conditions and maturity of the legumes.

(b) Colour characteristics of the chickpea flour

Micronization temperatures and seed tempering moisture conditions were found to influence the colour of chickpea flour (Table 3.2), however, effects were minimal when samples were tempered to 15% seed moisture and heated to 115°C to 150°C. From visual observation, the colour of chickpea flour was lighter with low micronization temperature and low seed moisture level. To confirm the visual appearance, at the same micronization conditions, Flour from untempered chickpea seed had higher L* values (lightness value) than the L* values of flour from seed tempered 15% and 22% seed moistures, respectively. The a* value, which measures the redness (+), of flour from seed micronized at 165ºC was found to be significantly (p<0.05) higher than non-micronized treatments and those flour from micronized seed to reach a lower temperature (115, 130, 150ºC) at corresponding moisture. The low a* value, which indicates a light red, showed that low seed moisture corresponded to a lower a* value. The b* value, an indicator of yellowness (+), showed the flour tended to be more bright in yellow when micronization to 115ºC was applied to 22%, 15% seed moisture and seed without tempering, respectively, whereas there were no significant (p<0.05) effect of seed tempering moisture and micronization on b* value of chickpea flour from micronized at 150ºC and 165ºC was observed.

Table 3.2 Colour of chickpea flour based on the CIE system (L* = lightness; a* = redness; b* = yellowness). Data represent the mean ± one standard deviation (n = 2).

Treatment	CIE colour		
	L*	a*	b*
Untempered			
Non-micronized	85.60 ± 0.47^{ab}	4.79 ± 0.05^{f}	22.76 ± 0.56^{e}
Micronized to 115^{o}C	86.10 ± 1.01^{a}	4.88 ± 0.19^{f}	23.06 ± 1.14^{de}
Micronized to 130^{o}C	85.72 ± 0.55^{ab}	4.84 ± 0.22^{f}	23.14 ± 0.96^{de}
Micronized to 150^{o}C	85.16 ± 1.04^{abc}	5.02 ± 0.23^{f}	22.84 ± 0.79^{e}
Micronized to 165^{o}C	83.60 ± 0.69^{d}	5.83 ± 0.44^{c}	23.48 ± 0.79^{de}
Tempered to 15% seed moisture			
Micronized to 115^{o}C	85.65 ± 0.72^{ab}	5.13 ± 0.08^{def}	24.74 ± 0.23^{bc}
Micronized to 130^{o}C	84.98 ± 1.37^{abcd}	5.08 ± 0.05^{ef}	24.24 ± 0.18^{bcd}
Micronized to 150^{o}C	84.07 ± 0.86^{cd}	5.62 ± 0.23^{cde}	23.61 ± 0.26^{cde}
Micronized to 165^{o}C	80.48 ± 1.30^{e}	7.10 ± 0.24^{b}	23.85 ± 0.37^{bcde}
Tempered to 22% seed moisture			
Micronized to 115^{o}C	84.42 ± 0.69^{bcd}	5.96 ± 0.31^{c}	26.98 ± 0.56^{a}
Micronized to 130^{o}C	83.98 ± 0.91^{cd}	5.67 ± 0.81^{cd}	24.97 ± 0.29^{b}
Micronized to 150^{o}C	80.08 ± 0.61^{e}	7.11 ± 0.43^{b}	23.30 ± 0.97^{de}
Micronized to 165^{o}C	76.73 ± 0.38^{f}	7.87 ± 0.37^{a}	23.18 ± 0.46^{de}

$^{a\text{-}f}$ Means within the same column with the same letter are not significantly different (p>0.05)

Overall, the colour of chickpea flour based on the CIE system showed that there was no significant (p<0.05) difference of L* and a* values for chickpea flour from non-micronized seed, flour from untempered chickpea seed when micronized to reach 115ºC, 130ºC, 150ºC, and flour from seed tempered to 15% seed moisture before micronized to 115ºC and 130ºC. No effect of micronization temperature (p<0.05) on b* values of flour from untempered and seed tempered to 15% seed moisture was found in this study.

From this study, it can be seen that colour of chickpea flour stayed very similar for the flour from non-micronized sample, untempered and tempered to 15% seed moisture content at corresponding micronization temperatures. There was no effect of micronization on flour colour under these seed tempering moistures and micronization conditions which might be due to the low initial seed moisture prior to micronization (untempered (7% moisture) and tempered to 15% seed moisture) and the micronization time was short, thus chickpea seeds did not receive sufficient heat to burn the seed coat or allow the Maillard browning reaction to occur.

The overall darkening effect and relationship between the lower L* value and higher seed tempering moisture level observed in chickpea flour as a result of micronization is presumed to be due to changes in the colour of the seed coat. Similar results were found by Mwangwela et al. (2007) when cowpea seed with 41% initial seed moisture was micronized to final surface temperatures of 130 and 170ºC. They reported that cowpea flour from the 170ºC micronized seeds was the darkest (lowest L* value) and it had showed the highest redness (highest a* value) resulting in greater brown colour. Arntfield et al. (1997) found that hull on green lentils seed with 33% initial seed moisture micronized to 138ºC was less bright, more red and slightly less yellow than non-micronized lentil seed. Moreover, colour of lentils was found to be significantly affected by seed tempering moisture by Arntfield et al. (1997). At the same micronization temperature, there was a significant decrease in the lightness (L*) as seed tempering moisture level increased from 25% to 29% and then to 33% moisture. The a* value was significantly higher for lentils seed tempered to 33% compared to 25% seed moisture. The lentil seeds tempered to 33% moisture had a significantly lower b* value than those of tempered to 25 and 29% (Arntfield et al., 1997). However, Mwangwela et al. (2007) demonstrated that in cowpea flour, the b* value (yellowness) increased as micronization temperature of seed increased contrasting in this study which found that b* value was higher when seed moisture content increased in this study (at lower micronization temperatures).

(c) Gelatinized starch content

Starch is a polysaccharide, which accumulates in the form of starch granules and is composed of two polymers, amylose and amylopectin. A linear polysaccharide chain consisting of (1→4)-linked α-D-glucopyranosyl units is known as amylose. Amylopectin is a highly branched polysaccharide composed of (1→4)-linked α-D-glucopyranosyl units with occasional α-D-(1→6) linkages which provide branching (BeMiller & Huber, 2008). In the presence of heat and excess moisture, the hydrogen bonds holding the starch together weaken, allowing water to penetrate into the starch molecules, causing starch granules to swell resulting in the loss of crystalline structure (BeMiller & Huber, 2008). Changes of microstructure of the seed from heating (i.e. precooking) affected starch functionality.

It was observed in the present study that tempering played an important role in the resulting percentage of starch gelatinization following the micronization process (Table 3.3).

Table 3.3 Gelatinized starch content of chickpea flour. Data represent the mean ± one standard deviation (n = 2).

Treatment	Gelatinized starch (%)
Untempered	
Non-micronized	nd
Micronized to 115°C	nd
Micronized to 130°C	nd
Micronized to 150°C	nd
Micronized to 165°C	nd
Tempered to 15% seed moisture	
Micronized to 115°C	nd
Micronized to 130°C	nd
Micronized to 150°C	nd
Micronized to 165°C	nd
Tempered to 22% seed moisture	
Micronized to 115°C	8.20 ± 1.71^{d}
Micronized to 130°C	18.52 ± 2.52^{c}
Micronized to 150°C	24.43 ± 4.09^{b}
Micronized to 165°C	33.96 ± 2.24^{a}

$^{a\text{-}d}$ Means within the same column with the same letter are not significantly different (p<0.05)
nd = not detected

Gelatinized starch content, found only in flour from micronized chickpea seed with 22% initial seed moisture, ranged from 8.20-33.96%, where the percentage was found to increase (p<0.05) as the micronized temperatures of seed was raised (Table 3.3). In all other flour treatments, no gelatinized starch was detected. Since moisture is necessary for the removal of amylose and swelling of the granules during starch gelatinization, the need to have sufficient moisture content in the seed during the gelatinization process is important (Arntfield et al., 1997; Nielsen, 1998).

According to Fasina et al. (2001), a low level (<10%) of gelatinized starch was found in legumes (kidney beans, green peas, black beans, lentils and pinto beans) flour which these seeds were micronized to reach 140ºC at low (<10%) initial seed moisture content. Sufficient moisture is required for heat transfer, chemical and structure changes. These caused starch gelatinization and protein denaturation of tempered heat-treated seed when heated to 130ºC and 170ºC with 41% moisture (Mwangwela et al., 2007). Similar results to the present findings were reported for lentils by Arntfield et al. (1997), where starch gelatinization increased from 42 % to 52%, and then to 70%, as the tempering levels increased from 25%, 29%, and 33% moisture, respectively. Moreover, Arntfield et al. (2004) reported that the amount of gelatinized starch was significantly (p<0.05) higher for pea flour from seed tempered to 24 and 30% moisture and micronized to 115ºC, with the pea flour from seed tempered to 30% having the highest value whereas, there was no gelatinized starch found in the untreated pea flour.

(d) Lipoxygenase activity

Lipoxygenase activities of the Kabuli chickpea seed (seed coat present) and the various flours after storage were measured (Table 3.4). The seed and the flour were vacuum packaged in polyethylene bags and stored at 4ºC. Sessa (1979) explained that oxidization of polyunsaturated fatty acids to aldehydes and alcohols can occur because of lipoxygenase. This could lead to off-flavours in legume protein products. The activity of this enzyme plays an important role for optimizing shelf-life or utilization of various food products, including meat products. Lipoxygenase activity of fresh ground chickpea flour from non-micronized seed was found to be 3.37×10^5 units/g of protein. There was a significant (p<0.05) decline of about 60% in lipoxygenase activity (to 1.98×10^5 units/g of protein) due to storage of the non-intact seed for flour from non-micronized seeds ground and stored for 2 years at 4ºC in vacuum packaged polyethylene bags.

Table 3.4 Lipoxygenase activity of chickpea flour. Data represent the mean ± one standard deviation (n = 2).

Treatment	Lipoxygenase activity ($\times 10^5$ units/g of protein)
Untempered	
Fresh ground non-micronized	3.37 ± 0.18^a
Non-micronized	1.98 ± 0.10^b
Micronized to 115ºC	1.12 ± 0.09^c
Micronized to 130ºC	nd
Micronized to 150ºC	nd
Micronized to 165ºC	nd
Tempered to 15% seed moisture	
Micronized to 115ºC	nd
Micronized to 130ºC	nd
Micronized to 150ºC	nd
Micronized to 165ºC	nd
Tempered to 22% seed moisture	
Micronized to 115ºC	nd
Micronized to 130ºC	nd
Micronized to 150ºC	nd
Micronized to 165ºC	nd

[a-c] Means within the same column with the same letter are not significantly different (p<0.05)
nd = not detected

The lipoxygenase enzyme accelerates oxidization of polyunsaturated fatty acids since lipoxygenase is an iron-containing enzyme. Iron (Fe^{2+}) steals a hydrogen atom from an unsaturated fatty acid and adds oxygen to the fatty acid creating hydroperoxides and free radicals (Sanz et al., 1994; Robinson et al., 1995). Although there was lipoxygenase activity present in chickpea flour from untempered seed which was micronized to 115ºC, 1.12×10^5 units/g of protein, no activity was found in any other treatments possibly due to the storage time and micronization conditions since lipoxygenase enzyme can be destroyed by heat. Moreover, since lipoxygenase enzyme is a protein, lack of lipoxygenase enzyme indicates some changes to protein fraction in the flours, therefore more testing is needed.

3.4.2 Functional properties of chickpea flours

(a) Water holding and oil absorption capacity

The water holding and oil absorption capacity of chickpes flours (dry weight basis) are presented in Table 3.5, Figure 3.1 and Figure 3.2. WHC and OAC are considered important attributes of a binder for use in a comminuted meat product.

Table 3.5 Water holding and oil absorption capacity of chickpea flour (dry weight basis). Data represent the mean ± one standard deviation (n = 2).

Treatment	WHC (g/g)	OAC (g/g)
Untempered		
Non-micronized	0.88 ± 0.02^{g}	0.90 ± 0.04^{cd}
Micronized to 115°C	0.94 ± 0.02^{fg}	0.98 ± 0.20^{cd}
Micronized to 130°C	1.01 ± 0.04^{ef}	0.98 ± 0.20^{cd}
Micronized to 150°C	1.10 ± 0.02^{cde}	0.91 ± 0.19^{cd}
Micronized to 165°C	1.13 ± 0.02^{cde}	0.91 ± 0.14^{cd}
Tempered to 15% seed moisture		
Micronized to 115°C	1.07 ± 0.02^{def}	1.23 ± 0.18^{b}
Micronized to 130°C	1.11 ± 0.01^{cde}	1.06 ± 0.22^{bc}
Micronized to 150°C	1.20 ± 0.01^{bc}	0.80 ± 0.01^{d}
Micronized to 165°C	1.19 ± 0.04^{bcd}	0.77 ± 0.01^{d}
Tempered to 22% seed moisture		
Micronized to 115°C	1.29 ± 0.01^{b}	1.53 ± 0.13^{a}
Micronized to 130°C	1.49 ± 0.05^{a}	1.70 ± 0.09^{a}
Micronized to 150°C	1.61 ± 0.08^{a}	1.63 ± 0.04^{a}
Micronized to 165°C	1.59 ± 0.14^{a}	1.51 ± 0.09^{a}

[a-f] Means within the same column with the same letter are not significantly different (p<0.05)

The ability of a product to associate with water under conditions where water is limited is represented by water holding capacity (Kaur & Singh, 2005). The oil absorption capacity is an essential factor of chickpea flour for consideration for use as an binder/extender in comminuted meat formulations, as oil improves the mouth feel and retains the flavour of the final product (Kaur & Singh, 2005; Mwangwela et al., 2007).

Overall, flour from micronization of chickpea seed with/without tempering of seed significantly ($p<0.05$) improved its WHC except that of flour from untempered chickpea seed which when seed micronized to 115ºC showed a similar result as chickpea flour from non-micronized seed, 0.94 and 0.88 g/g dry weight basis, respectively. Similar results to these values were shown for WHC of chickpea flour from non-micronized seed. The WHC of Kabuli chickpea flour from non-micronized seed was found to be 0.81 g/g (Sanjeewa, 2010) which is similar to the results of WHC. Moreover, Kaur & Singh (2005) found that WHC of untreated Kabuli chickpea flour was 1.33 g/g which was higher than the result from this study. This discrepancy may be due to growth conditions and crop year which could affect the protein content.

Overall, there was minor differences in WHC of flours from untempered treatments and those tempered to 15% seed moisture, when compared at the same micronization temperature, with small increases in WHC at higher micronization temperatures. On the other hand, tempering to 22% seed moisture, compared at corresponding temperatures, resulted in flours with significantly ($p<0.05$) higher WHC than from seeds that were untempered or tempered to 15% initial seed moisture, and larger effects on WHC with increased micronization temperature. Those flour from seed tempered to 22% initial seed moisture and micronized to temperatures of 130, 150 and 165°C had significantly higher WHC compared to other chickpea flour treatments.

In general, the WHCs observed for flours from micronized chickpea seed were higher than those from chickpea flour from non-micronized seed, which is presumed to be due to the opening of the starch granule which lead to an increase water binding. The exposure of amylose and amylopectin polymers from starch granule resulting from heat-induced starch gelatinization. Moreover, the protein possible effect separately since the exposure of more charged surface residues are caused by the heat-induced unfolding of proteins (Damodaran, 2008; Aguilera et al., 2009; Der, 2010; Ma et al., 2011). In addition, an increase in WHC might be due to the physical changes of chickpea seeds caused by micronization resulting in seed cracking which allows water to penetrate into the seed (Kayitesi et al., 2013). Mwangwela et al. (2007) reported that high micronization (41% seed moisture and micronized to 170ºC) significantly improved WHC of cowpea flour compared to 130ºC (41% seed moisture) and non-micronized treatments. The WHC of micronized lentil (15% seed moisture and micronized to 135ºC) was about 27% higher (1.0-1.1 g/g) than that of non-micronized lentil (0.7-0.8 g/g) (Der, 2010).

Tempering chickpea seed to reach 22% moisture before micronization enhanced OAC of chickpea flour by about 76% when compared with chickpea flour from non-micronized seed, and 68% when compared with chickpea flour from seed tempered to 15% initial seed moisture and micronized (Table 3.5). The OAC of chickpea flour from 22% seed moisture was found to be significantly (p<0.05) higher (1.53-1.70 g/g) than those at lower seed moisture levels, such as at 15% seed moisture (0.77-1.23 g/g) and untempered (0.91-0.98 g/g) (Table 3.5). The OAC of chickpea flour from non-micronized seed (0.90 g/g) was similar to chickpea flour from micronized seed to reach 115ºC, 130ºC, 150ºC and 165ºC after the seed was immersed in water to reach 15% seed moisture (0.91-0.98 g/g). There was no significant (p<0.05) effect found as a function of temperature for OAC when untempered and tempered to 22% chickpea seed moisture were micronized, whereas OAC significantly (p<0.05) declined in treatments tempered to 15% seed moisture with higher micronization temperatures, 150 and 165ºC.

According to Kaur & Singh (2005) the capacity of Kabuli chickpea flour to bind oil was 1.24 g/g which is higher than the results from this study and from Sanjeewa (2008), 0.90 and 0.87 g/g respectively, which were similar. The results from this study showed that no differences of OAC were found when comparing various micronization treatments of seed with similar initial seed moisture content which were comparable to the OAC results of cowpea flour (41% seed moisture and micronized to 130 and 170°C) from the study of Mwangwela and his colleagues (2007) and lentil flour (15% seed moisture and micronized to 135ºC) from the study of Der (2010) in which tempering increased OAC of legume flour.

According to Arntfield et al. (2001), micronized lentil seed had a more open structure which was observed by using scanning electron microscopy. Micronized cowpea seed was less dense than non-micronized; moreover fissures in the seed coats and cotyledons was reported, which might play an essential role in higher water absorption, higher oil holding capacity, and higher leaching losses when soaked in water (Fasina et al., 2001; Mwangwela et al., 2007). Moreover, increased OAC has been attributed to enhanced hydrophobic properties of proteins and the presence of non-polar amino acid side chains which bind with the aliphatic chain of fats (Kinsella, 1981; Mwangwela et al., 2007; Ma et al., 2011). Based on this suggestion, it could be inferred that the need to have a high moisture content in the seed prior to micronization is important since more non-polar residues from the interior of protein molecules were exposed after micronization at sufficient seed moisture content. The starch granules are opened due to sufficient moisture during heating; causing water and/or oil to more easily enter the granule and cause gelatinization of starch. Moreover, the high initial seed moisture with high micronization temperature may have induced greater porosity allowing greater entrapment of water and/or fat compared to the non-micronized and micronized at insufficient seed moisture.

(b) Pasting properties of chickpea flours

Pasting curves and corresponding rapid visco analyzer (RVA) parameters (Table 3.6) show differences among chickpea flours due to the effect of seed tempering moistures and micronization temperatures. Pasting temperature provides an indication of the minimum temperature needed to cook the flour (including the starch), and relates to the water entering the starch granule and hydrating the amylose and amylopectin molecules, leading to swelling and initiating the breakdown of the granule or gelatinization. Peak viscosity shows the maximum viscosity when the most granules are swollen which relates to water holding capacity of the starch, while breakdown gives an idea of starch paste stability and final viscosity represents the ability of flour to form a viscous paste or gel after cooking and cooling (Kaur & Singh, 2005; BeMiller & Huber, 2008; Sanjeewa, 2010; Singh et al., 2010; Tiwari et al., 2011).

Table 3.6 Pasting properties of chickpea flours. Data represent the mean ± one standard deviation (n = 2).

Treatment	Peak 1 (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Peak Time (Min)	Pasting temperature (ºC)
Untempered					
Non-micronized	99.21 ± 4.27^{de}	6.67 ± 0.99^{c}	110.42 ± 4.97^{d}	6.69 ± 0.22^{ab}	71.68 ± 0.34^{ef}
Micronized to 115ºC	115.07 ± 2.88^{bc}	7.58 ± 0.97^{c}	127.71 ± 5.01^{bc}	6.67 ± 0.24^{ab}	70.63 ± 0.68^{f}
Micronized to 130ºC	131.46 ± 0.58^{a}	10.72 ± 3.34^{bc}	141.78 ± 1.99^{ab}	6.61 ± 0.22^{ab}	71.00 ± 1.15^{ef}
Micronized to 150ºC	135.99 ± 6.35^{a}	17.43 ± 5.71^{ab}	149.75 ± 2.86^{a}	6.47 ± 0.49^{ab}	71.66 ± 1.74^{ef}
Micronized to 165ºC	137.76 ± 8.28^{a}	24.69 ± 9.07^{a}	148.19 ± 4.11^{a}	6.36 ± 0.47^{b}	71.80 ± 0.54^{ef}
Tempered to 15% seed moisture					
Micronized to 115ºC	129.21 ± 6.43^{ab}	8.68 ± 1.37^{c}	145.39 ± 5.21^{a}	6.87 ± 0.13^{ab}	73.60 ± 2.41^{de}
Micronized to 130ºC	113.21 ± 4.04^{cd}	11.22 ± 2.50^{bc}	136.81 ± 2.12^{ab}	6.97 ± 0.08^{a}	75.82 ± 2.34^{d}
Micronized to 150ºC	101.44 ± 5.88^{cde}	10.79 ± 0.92^{bc}	127.65 ± 6.08^{bc}	6.99 ± 0.03^{a}	80.66 ± 2.62^{bc}
Micronized to 165ºC	94.38 ± 12.79^{ef}	10.53 ± 5.54^{bc}	117.57 ± 9.80^{cd}	6.78 ± 0.12^{ab}	83.20 ± 2.36^{b}
Tempered to 22% seed moisture					
Micronized to 115ºC	84.32 ± 2.56^{f}	12.28 ± 1.56^{bc}	110.00 ± 2.46^{d}	6.97 ± 0.04^{a}	78.86 ± 0.94^{c}
Micronized to 130ºC	55.42 ± 8.38^{g}	10.18 ± 2.49^{bc}	84.29 ± 8.42^{e}	6.99 ± 0.03^{a}	82.18 ± 1.21^{b}
Micronized to 150ºC	37.53 ± 6.56^{h}	6.31 ± 1.21^{c}	59.93 ± 8.09^{f}	7.00 ± 0.00^{a}	88.48 ± 1.40^{a}
Micronized to 165ºC	33.57 ± 12.13^{h}	5.01 ± 1.66^{c}	52.47 ± 15.18^{f}	7.00 ± 0.00^{a}	88.47 ± 1.82^{a}

$^{a\text{-}f}$ Means within the same column with the same letter are not significantly different (p<0.05)

Pasting temperature of chickpea flour from non-micronized seed found in this study was 71.7ºC while others found that pasting temperature of chickpea flour ranged from 73.1-75.2ºC (Kaur & Singh., 2005) to 75.0-87.1ºC (Singh et al., 2010). The pasting temperature of chickpea flour from untempered and micronized seed ranged from 70.63 to 71.80ºC which was not significantly ($p<0.05$) different from chickpea flour from non-micronized seed. In the present study, the pasting temperature of the flours increased when seeds were tempered to achieve higher moisture, 73.60 to 83.20ºC for those tempered to 15% seed moisture, and 78.86 to 88.47ºC for seed moisture of 22% level. Moreover, in case of micronization temperature (relative to the same tempering conditions), pasting temperature tended to be higher when micronization temperature increased. At the level of 15% and 22% initial seed moisture, pasting temperature of chickpea flour from seed micronized to 150 and 165ºC was significantly ($p<0.05$) higher than flour from seed micronized to 115 and 130ºC at corresponding moistures.

Peak viscosity and final viscosity of chickpea flour from non-micronized seed was 99.21 and 110.42 RVU, respectively. All flour treatments from untempered seed followed by micronization showed a gradual increase in peak viscosity and final viscosity with an increase in temperature; whereas, when tempering (moisture) was provided to the seeds, peak viscosity and final viscosity acted in opposite direction with temperature (Table 3.6). The peak viscosity and final viscosity of chickpea flours from micronized seed decreased as seed moisture increased (15% and 22% initial seed moisture) compared at corresponding temperatures. There might be two main factors affecting the results of this study. The increase in viscosity from RVA when untempered seed was micronized may occur since less water in seeds lowers the leaching of amylose out of the granules. Upon reheating by RVA (with water added), the starch granules are swollen and opened up and the amylose can easily leach out of granule contributing to an increase in viscosity. In case of high seed moisture, more water helps stabilize protein via hydrogen bonding therefore less denaturation of protein and more amylose leaves granules, which upon reheating, there is amylose chain breakdown which leads to a decrease in viscosity. Moreover, when considered at the same seed moisture content (15% and 22%), when higher micronized temperature was delivered to the seeds, the formation of amylose, amylopectin and/or lipids due to hydrogen bonding occurs resulting in increased the physical crosslink of starch which affect the reduction of swelling causing reduce in viscosity during reheated by RVA (Mwangwela et al., 2007).

3.4.3 Correlation coefficients for chickpea flour properties

There was a significant ($p<0.001$) positive correlation between gelatinized starch and water holding capacity (r = 0.89), oil absorption capacity (r = 0.76), and peak viscosity (r = 0.90). Gelatinization of starch is a process that breaks down the intermolecular bonds of starch molecules allowing the hydrogen bonding sites to engage more water, leading to an increase in water holding capacity and peak viscosity. In addition, more water helps stabilize the protein via hydrogen bonding which lead to less denaturation of protein affecting the higher oil absorption capacity since protein acts as an emulsifier between water and oil (Sanjeewa, 2008).

The significant negative relationship was found between peak viscosity, peak time (r = -0.57, $p<0.01$) and pasting temperature (r = -0.92, $p<0.001$). The highest WHC of the starch molecules in the granule can be indicated by peak viscosity. When chickpea flour displayed greater peak viscosity, the flour also required lower minimum temperature to cook (lower pasting temperature) and lesser time to reach this peak which might due to the fact that starch was already partially gelatinized.

3.5 Conclusion

Kabuli chickpea seed was sub-divided into 3 groups: a) untempered seed (7% moisture); b) seed tempered to a 15% moisture level; and seed tempered to a 22% moisture level. All groups were micronized to reach four different surface temperatures: 115, 130, 150, and 165^{o}C. Once micronized, seeds were milled to yield a flour, and then analyzed based on changes to their physical, chemical and functional characteristics. Results were compared with the chickpea flour from non-micronized seed. Chickpea flour which was used in this study contained 19.92% protein, 7.39% fat, 2.74-2.87% ash (dwb) and pH ranged from 5.96 to 6.14.

In order to create chickpea flours from micronized and/or tempered seed which have different properties from flour from non-micronized seed, the high seed tempering moisture level was requried at low micronization temperature. Results of this study indicate that micronization temperatures and tempering conditions of seed influenced the colour of chickpea flour. The colour of chickpea flour was darker with higher seed moisture and higher micronization temperature that might be due to the color of seed coat after heated by micronization. The effect of micronization temperature on yellow colour of flour from untempered and tempered to 15%

seed moisture before micronized was not found in this study. Whereas, the bright yellowness of chickpea flour was found when micronization to 115ºC was applied to 22% seed moisture.

Increasing micronized temperature when 22% seed moisture was reached significantly increased starch gelatinization from 8.2 to 34.0%, which lead to higher WHC and OAC. For the pasting properties final viscosity decreased and pasting temperatures increased with increasing micronization temperatures of seed when chickpea seed was tempered to 15 and 22% moisture which might due to the effect of gelatinized starch. Micronization also was found to reduce lipoxygenase activity in chickpea flour. The lipoxygenase activity of fresh ground flour from non-micronized chickpea seed was found to be 3.37×10^5 units/g of protein and 1.98×10^5 units/g of protein in flour from seeds ground and stored for 2 y. Values for lipoxygenase activity for untempered chickpea flour which was micronized to 115ºC was 1.12×10^5 units/g of protein whereas, no lipoxygenase activity was found in any other micronized treatments.

3.6 Connection to the next study

The overall goal of this project was to study chickpea flour properties to find an optimum condition, as it will be used as a binder in a model low-fat pork bologna. A second study was therefore carried out to examine the usefulness of 10 chickpea flour treatments from study 1 as a binder, as an alternative to wheat flour. Various textural and sensory properties of the resulting bolognas were compared. The chickpea flour from seed which was micronized to 165ºC (untempered, tempered to 15% and 22% seed moisture) was not included in study 2 as it showed a strong undesirable off flavour from preliminary sensory trials.

4. Study 2: Effect of flours produced from micronized chickpea seeds on the physical, chemical, and sensory properties of a model low-fat pork bologna product

4.1 Abstract

The effect of seed tempering moisture (7, 15 and 22% moisture) and seed micronization temperature (115, 130, 150^{o}C) of chickpea flour (5% addition level) on the physical, chemical, and sensory attributes of low-fat (<10%) pork bologna was investigated. The flour treatments from seed micronized to 165^{o}C were not included as part of this study based on preliminary sensory work which showed a strong off-flavour and undesirable taste. No significant (p<0.05) differences of cook loss (0.3-0.4%) and expressible moisture (14.1-15.3%) were found among all bologna with the addition of chickpea flour, whereas bologna containing wheat flour had significantly (p<0.05) lower cook loss (0.3%) and expressible moisture (10.0%) than with the addition of chickpea flour from non-micronized seed. The bologna with 5% wheat flour showed significantly (p<0.05) lower purge losses (3.2%) than the control bologna (no binder, 5.4% purge), and all samples containing flour from micronized chickpea seed (4.0-4.8%), while addition of chickpea flour from non-micronized seed showed the lowest purge loss (3.9%) among all chickpea bologna treatments. Bologna containing chickpea flour from micronized seed were slightly more yellow in colour (CIE system) than bologna prepared with wheat flour and no binder. Bologna was further evaluated by instrumental texture profile analysis (TPA) which showed that chickpea flour from seed tempered to 15% and 22% seed moisture did not improve TPA hardness and chewiness of the bologna (compared with the addition of untempered flour) when the seed was micronized to reach 130^{o}C and 150^{o}C (p<0.05). Whereas bologna produced with chickpea flour from seed tempered to 22% following 115°C micronization had significantly (p<0.05) higher firmness as determined by both instrumental texture and sensory evaluation by 12 trained panelists. From sensory evaluation results, there was no significant (p<0.05) effect of micronization and seed tempering moisture conditions on colour, juiciness, saltiness and flavour intensity among all bologna containing chickpea flour. Bologna prepared with flour from non-

micronized chickpea seed had the highest foreign flavour, and the lowest flavour desirability and overall accceptability score, whereas bologna with the addition of chickpea flour from seed micronized to 150^{o}C without tempering and from seed tempered to 22% following 115^{o}C micronization were found to have higher flavour desirability and overall acceptability scores than bologna with added wheat flour.

4.2 Introduction

Consumer eating habits have changed in recent years, as consumers have become more health conscious. Development of healthier meat products involves the decreased content of fat, salt, cholesterol and calories (Pietrasik & Janz, 2010). In particular, the meat industry has attempted to reduce fat content in meat products for health reasons. The limit of total fat intake to be less than 30% of total calories has been proposed by health organizations all over the world. The lower intake of saturated fatty acids and cholesterol has been advised to prevent cardiovascular heart disease (Pietrasik & Janz, 2010; Sanjeewa, 2010). Reducing the fat in the raw material is the most efficient way to produce low fat meat products. However, problems including undesirable texture, reduced production yields, soft mushy interiors, rubbery skin formation, excessive purge, and changes in sensory qualities after cooking or reheating can happen when fat is directly substituted by water (Claus et al., 1989; Pietrasik & Janz, 2010).

Non-meat ingredients derived from various plant sources such as soy, beans, peas, lentils, chickpea have been utilized as fillers, binders, emulsifiers or extenders in meat systems. These materials can cut down the cost and serve as functional ingredients while improve or at least maintaining nutritional and sensory qualities of the end product that consumers expect (Pietrasik & Janz, 2010; Sanjeewa, 2010). Generally plant proteins are cheaper than meat proteins, and their substitution results in lowering the cost of meat products (cost reduction and volume increase).

Chickpea (*Cicer arietinum* L.) is the second largest pulse crop in the world based on the cultivated area and production. Canadian chickpea is widely grown in Saskatchewan and Alberta, which produce 80% and 20%, respectively (Saskatchewan Pulse Growers, 2013). Chickpea provides energy, dietary fibre, proteins, minerals and vitamins required for human health. High nutrient foods have been in demand from customers whereas industries want to bring down the cost. Therefore, chickpea could provide new opportunities for the Canadian pulse and meat industries.

The purpose of this study was to improve properties of low-fat pork bologna and extend the utilization of chickpea flour along with micronization and seed tempering conditions in meat processing. Since micronization temperatures and tempered conditions of chickpea seeds affect physical and chemical properties of chickpea flour (study 1), using chickpea flour as a binder in a model low-fat pork bologna product was investigated. The physical properties and sensory characteristics of the bolognas were explored.

4.3 Materials and methods

Chilled fresh lean pork leg muscles were obtained from a commercial meat packing company and kept at 1ºC. Pork backfat was kept frozen at -30ºC then thawed at 1ºC for 24 h. After that lean pork leg muscles and pork backfat were ground separately for two times: first through a plate with 6.5 mm diameter holes (3/8” perforated disc), then through a plate with 3.9 mm diameter holes, 3/16” perforated disc (Biro Grinder model AMFG-24, Marblehead, OH, USA), vacuum packaged, and kept at 1ºC prior to use. Non-meat ingredients which were regular salt, Griffith prague powder (6.4% sodium nitrite, 93.6% sodium chloride, Griffith Laboratories, Scarborough, ON, Canada), sodium tripolyphosphate (Unipac Packaging Products Ltd, Edmonton, AB, Canada), sodium erythorbate (Unipac Packaging Products Ltd, Edmonton, AB, Canada), german weiner seasoning (Hela Spice Canada Inc, Uxbridge, ON, Canada) and chickpea/wheat flour were weighed and kept at 1ºC for at least 12 h prior to use. There were 10 chickpea flour treatments involved in this study as a binder: chickpea flour from non-micronized seed, untempered seed, seed tempered to 15% and 22% seed moisture with micronization to 115°C, 130°C and 150°C. Two other treatments included a no binder control and one with wheat flour, the industry standard for binders in bologna. Binders replaced 5% of the meat in the no binder treatment and met Canadian regulations for minimum total protein content (11% protein). The experiment was performed in triplicate.

4.3.1 Preparation of low-fat pork bologna

Each bologna treatment was formulated to produce meat batters with 11% protein, comprised of a combination of meat and chickpea flour protein (in compliance with Canadian regulations for minimum meat and total protein content) and 10% fat. The level of meat and pork backfat varied between replications due to the amount of fat and protein in that meat source. Lean pork leg muscles and pork back fat used in this study contained 21.33 ± 0.11, 3.53 ± 0.71% protein and 3.42 ± 1.08, 79.33 ± 1.15% fat, respectively. Non-meat ingredients included 1.7% salt, 0.30% prague powder (6.4% w/w sodium nitrite), 0.20% sodium tripolyphosphate, 0.10% sodium erythorbate and 0.48% german weiner seasoning, and 5.0% of chickpea/wheat flour (dry basis). Water was adjusted based on changes in flour weight. An example of the formulations used in this study is given in Table 4.1.

To ensure desirable texture and consistency of product (Aberle et al., 2001), temperature of the meat batter was examined at every stage during the process. Initial temperature of the meat was 1.8 to 3.2°C. The finely ground meat, salt, prague powder, sodium tripolyphosphate, and 1/3 of ice/water were mixed by chopping in a bowl chopper (35-L table top bowl chopper, model #84181D, Hobart, Troy, Ohio, USA), with knife speed No. 4 and bowl speed No. 2 for 2 min, then pork backfat, seasoning, sodium erythorbate, wheat flour/chickpea flour and another 2/3 of ice/water were added and chopped for 2 min. The total chopping time was 4 min and emulsion temperature did not exceed 11°C. After that the batter was passed through an emulsion mill (Type 1E-75F, Alexanderwerk, Remscheid, Germany) twice. The final temperature of the emulsion did not exceed 18°C. The batter temperature before cooking ranged from 2.9 to 3.7°C. Then, the meat emulsion was placed in vacuum tumbler (Model VSM-150H., Glass, Frankfurt, Germany), with a vacuum pulled for 3 min, and the vacuum released. This process was repeated twice to remove the air trapped in the meat batter matrix. Temperatures of the meat batter before and after chopping, and prior to stuffing were recorded. The batter was then transferred to a piston stuffer (Model EL-20, Mianca Equipamientos Carnicos, S.L., Barcelona, Spain) and stuffed (~1 kg) into water impermeable pre-weighed plastic casings (63 mm stuff diameter, Walsroder KFS MATT red, Art.-no.: 40216663, CaseTech GmbH, Walsrode, Germany). The stuffed batter (chubs) were twisted by hand, clipped with aluminum clips, and washed to clean the outside of the casings.

Table 4.1 Low-fat pork bologna formulations containing various binders.

Binder type	Ingredient[1]			
	Pork leg meat (%)	Pork back fat (%)	Ice/water (%)	Flour (%)[2]
No binder	51.53	10.83	34.86	0.00
Wheat flour	46.87	10.51	34.38	5.46
Chickpea flour				
Untempered				
Non-micronized	46.87	10.51	34.47	5.37
Micronized to 115°C	46.87	10.51	34.60	5.24
Micronized to 130°C	46.87	10.51	34.65	5.19
Micronized to 150°C	46.87	10.51	34.74	5.10
Tempered to 15% seed moisture				
Micronized to 115°C	46.87	10.51	34.52	5.32
Micronized to 130°C	46.87	10.51	34.54	5.30
Micronized to 150°C	46.87	10.51	34.67	5.16
Tempered to 22% seed moisture				
Micronized to 115°C	46.87	10.51	34.21	5.63
Micronized to 130°C	46.87	10.51	34.38	5.45
Micronized to 150°C	46.87	10.51	34.62	5.22

[1]1.7% salt, 0.30% prague powder (6.4% w/w sodium nitrite), 0.20% sodium tripolyphosphate, 0.10% sodium erythorbate and 0.48% german weiner seasoning were added into every batch.
[2]% flour based on 5.0% dry weight basis.

Cooking of bologna was done by immersing the stuffed batter in an agitated water bath set at 75°C and cooked using a four-stage process schedule: 30 min at 50°C (initial water temperature), 30 min at 60°C, 30 min at 70°C, and then followed by cooking at 75°C until the final internal temperature reached 72°C (approximately 2 h). Immediately, cooked bologna was cooled down in ice-cold water (~4°C) for 1 h to prevent overcooking of the product and stored at 4°C until analyses. The procedure to produce bologna was performed in a refrigerated pilot plant (~4°C) at the Department of Food and Bioproduct Sciences, University of Saskatchewan.

4.3.2 Physical analysis of low-fat pork bologna

The physical properties of low-fat pork bologna containing chickpea flour (or wheat flour) was determined by the following methods.

(a) Cooking loss

The loss due to cooking was determined using three bologna chubs from each treatment. Each bologna chub was weighed immediately after cooked and chilled at 4°C for 24 h, and then the bologna was opened by cutting one side with a clean scissors and blotted by rolling over a paper towel. The percentage of cooking loss was estimated based on weight after cooking.

$$\text{Cook loss (\%)} = \frac{\text{Initial bologna weight (g) - Blotted weight (g)}}{\text{Initial bologna weight (g)}} \times 100 \qquad \text{(eq. 4.1)}$$

Initial bologna weight = weight of bologna after cooking and chilled at 4°C for 24 – (weight of casing + metal clips)

(b) Batter viscosity

After stuffing, some of meat batter was transferred into a 250-mL plastic cup which was used to measure the batter viscosity by using a Brookfield Synchro-Lectric viscometer (Model RVT; Brookfield Engineering, Stoughton, MA, USA). The viscosity was measured in triplicate at a speed of 10 rpm and using spindle number 7. The batter temperature was read. The batter viscosity was recorded after the spindle was rotated for 30 s.

(c) Expressible moisture

Expressible moisture of low-fat pork bologna was determined using a modified method from Sanjeewa (2008), in triplicate. Two pieces of Whatman No. 3 (5.5 cm in diameter) and one piece of Whatman No. 50 (7.0 cm in diameter) were folded into a thimble shape before being placed in a 50-mL plastic centrifuge tube. A bologna sample (1.5-2.0 g) was placed in the thimble and centrifuged for 15 min at $750 \times g$ (1900 rpm) at 4^{o}C using SH 3000 BK Rotor (Sorvall RC-6 Plus TM Superspeed Centrifuge, Thermo Fischer, Scientific). Expressible moisture was calculated as the following formula.

$$\text{Expressible moisture (\%)} = \frac{\text{Initial sample weight (g) - Final sample weight (g)}}{\text{Initial sample weight (g)}} \times 100 \qquad \text{(eq.4.2)}$$

(d) Purge loss

Purge loss during simulated retail storage of vacuum packaged bologna slices were investigated in duplicate for every treatment. The test is related to consumer acceptability of the product's appearance. Twelve slices of bologna (3 mm thickness each, 2 stacks of six per pack) were placed in a pre-weighed bag, vacuum packed, and stored in an upright position at 4^{o}C for 14 days of storage (Bloukas & Paneras, 1993) and the amount of liquid that collected inside the package during storage time was determined. The vacuum-packaged bologna was weighed before and after opening. For the weight after opening, the bologna and vacuum bag was blotted with paper towels before weighing. Purge loss was shown as percentage of initial weight.

$$\text{Purge loss (\%)} = \frac{\text{(Initial bologna - bag weight) - (Final bologna - bag weight)}}{\text{(Initial bologna - bag weight)}} \times 100 \qquad \text{(eq. 4.3)}$$

(e) Texture profile analysis

Texture profile analysis was determined by using TMS-Pro Texture Press (Food Technology Corp., Rockville, MD, U.S.A.) interfaced with a computer, using the supplied software (Texture Technologies Corp., Texture Lab Pro, version 1.13-002). Before testing, bologna samples were cut precisely into 35 mm diameter cores, trimmed to 25 ± 0.5 mm in height and then allowed to equilibrate to room temperature for 1 h. Eight cores were measured in total from each treatment (four from each of two chubs per treatment). The samples were compressed twice between parallel flat plates fixtures to 50% of their original height at a crosshead speed of 50 mm/min using a 250N capacity load cell (Hayes at al., 2005; Dong et al., 2007). The following parameters of bologna were obtained (Bourne, 1978):

- Hardness (the peak force of the first compression of the sample which also known as firmness)
- Cohesiveness (the degree to which the sample withstands a second deformation relative to how it behaved under the first deformation)
- Adhesiveness (the work needed to overcome the attractive forces between the surfaces of the sample and the surface of other materials with the sample comes in contact))
- Springiness (the degree to which a sample physically springs back after it has been deformed during the first compression)
- Chewiness (hardness × cohesiveness × springiness which is defined as the energy required to disintegrate a sample to a state of swallowing)

(f) Torsional geometry analysis

Torsional geometry is a method that is used to determine the firmness and elasticity of the product, in which true shear stress and shear strain were assessed. The method is based on the procedures of Foegeding (1990) and Meullenet et al. (1994). From each treatment, 12 core bologna samples (obtained from 2 chubs, 6 cores per chub) were cut to 28.7 mm in length and 19 mm in height (using a metal cork borer). Then samples were glued to two-slotted plastic styrene discs using cyanoacrylate glue (Locktite® 404 instant adhesive, Loctite Corp., Newington, CT). The samples were formed into a dumbbell shape with a 12.5 mm in minimum diameter by rotation against a shape rotating grinding wheel (Model KCI-24A2, Bodline Electric Co., Chicago, IL). After that, the dumbbell-shape samples were placed in a bottom torsion fixture (Gel Consultants, Inc., Raleigh, NC) attached to a Brookfield digital viscometer (Model DV-I+, Brookfield Engineering Laboratories, Inc., Stoughton, MA) in order to rotate at 2.5 rpm until the structural failure. Shear stress and shear strain at failure were recorded.

(g) Colour

The colour of bologna was evaluated in duplicate per treatment obtained from two chubs. A Hunterlab MiniScan XE colourimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used based on L^*, a^* and b^* dimensions from the CIE Lab system value with illuminant A and a 10º observer.

4.3.3 Chemical analysis of the cooked product

Proximate composition of the bologna was determined by following AOAC (1990) and AACC (1999) methods, which pH, total moisture, protein content, crude fat, total ash were evaluated. Refer to Section 3.3.3 for the detailed procedures.

4.3.4 Sensory evaluation

Trained panel study of low-fat pork bologna

The present study was accepted on ethical grounds (BEH # 12-159) by the University of Saskatchewan Behavioral Research Ethics Board. For overall quality, twelve formulations (the low-fat pork bologna containing chickpea flour from non-micronized seed, untempered seed, seed tempered to 15% and 22% seed moisture followed by micronization to temperatures of 115°C, 130°C and 150°C, wheat flour, and no binder treatment) were assessed for sensory characteristics by 12 panelists (staff and/or students from the Department of Food and Bioproduct Sciences). Initially, 16 people received a basic taste screening by identification of basic tastes (sweet, sour, salty, bitter, umami) at two different concentrations, followed by training sessions with bologna products to evaluate colour, initial firmness, chewiness, overall juiciness, saltiness, flavour intensity, foreign flavour and flavour desirability. After three weeks of training (20-30 min per day, 2 days per week), those panelists were asked to evaluate the representative samples of bologna treatments with 8-point scales and sensory terminologies (Appendix A). From 16 panelists, only 12 panelists were qualified to participate in the experimental bologna trial in order to evaluate sensory attributes of low-fat pork bologna containing chickpea flour. The panelists were chosen based the comparison between the sensory scores and the results obtained from texture profile analysis (TPA). Moreover one-way analysis of variance was used to assess the ability of panelists to consistently detect differences and to match samples which were the same.

The panelists were asked to evaluate a labeled reference sample (bologna produced with wheat flour) and 6 experimental samples at each sensory session. Therefore, two days of sensory evaluation were needed per replication in order to reach the total of 12 experimental samples. Samples were labeled with three-digit random codes. Five pieces per sample were placed into covered plastic cups, kept at 4^{o}C until served to panelists in random order. Water at room temperature and unsalted crackers were served to panelists to cleanse their palate between samples. The sample were prepared by slicing the 60 mm diameter bologna to 12.5 mm thickness and then manually cut using a dual scalpel blade knife to give 12.5 mm x 12.5 mm cubed samples. Both ends of low-fat bologna chubs were cut off and not used for the evaluation. There were 3 replications performed in this trained panel study. The panels were conducted in a sensory room with individual booths under red lighting for texture and flavour attributes; whereas, white lighting was applied for colour evaluation. Bologna samples were scored for colour (8 = extremely pinkish red, 1 = extremely brown), initial firmness (8 = extremely firm, 1 = extremely soft), chewiness (8 = extremely hard to chew, 1 = extremely easy to chew), overall juiciness (8 = extremely juicy, 1 = extremely dry), saltiness (6 = extremely salty, 1 = not detectable), flavour intensity (8 = extremely intense, 1 = extremely bland), flavour desirability (8 = extremely desirable, 1 = extremely undesirable), foreign flavour (8 = extremely intense, 1 = no foreign flavour), overall acceptability (8 = extremely acceptable, 1 = extremely unacceptable).

4.3.5 Statistical analysis

This study was repeated three times. Observed data were arranged into a Randomized Complete Block Design using Statistical Package for the Social Sciences (SPSS) software (SPSS 16.0, SPSS, Inc., Chicago, IL, USA). Variations contributed by meat materials used in each replicate were considered as block. Treatment and interaction between factors were included in the model. The comparison a between individual treatment means was assessed by the least significant difference (LSD) procedure. The significance was declared at $p<0.05$. For the trained sensory panel study, bologna treatment, replication, panelist, and interaction were included in the model. Means were analyzed and compared using the least significant difference (LSD) procedure of SPSS. The level of significance was set at $p<0.05$. Pearson correlation coefficients (SAS Institute, 2008) were determined among the various parameters tested ($p<0.05$).

4.4 Results and discussion

(a) Raw batter properties

The resistance of the meat emulsion to flow can be measured by batter viscosity. High batter viscosity leads to an increase in firmness and springiness of the finished product (Keever, 2011). If the viscosity is too low, handling issues during the pre-stuffing and stuffing processes may occur. To improve raw batter viscosity, plant binders such as wheat flour are typically added for their thickening effects (Sanjeewa et al., 2010).

Table 4.2 Effect of flour binders on the apparent viscosity of raw low-fat batters and batter temperature before cooking. Data represent the mean ± one standard deviation (n = 3).

Treatment	Viscosity (cPs) × 10^4	Batter temperature (°C)
No binder	6.18 ± 0.44^{b}	3.17 ± 0.51^{ab}
Wheat flour	8.55 ± 0.96^{a}	3.20 ± 0.72^{ab}
Chickpea flour		
Untempered		
Non-micronized	8.10 ± 1.52^{ab}	3.30 ± 0.89^{ab}
Micronized to 115°C	8.14 ± 1.13^{a}	3.03 ± 0.46^{ab}
Micronized to 130°C	7.42 ± 0.83^{ab}	3.43 ± 0.50^{ab}
Micronized to 150°C	8.00 ± 0.86^{ab}	3.57 ± 0.55^{ab}
Tempered to 15% seed moisture		
Micronized to 115°C	7.67 ± 0.70^{ab}	3.70 ± 0.50^{a}
Micronized to 130°C	8.13 ± 1.57^{a}	3.33 ± 0.12^{ab}
Micronized to 150°C	8.05 ± 0.90^{ab}	2.90 ± 0.79^{b}
Tempered to 22% seed moisture		
Micronized to 115°C	9.24 ± 1.14^{a}	3.50 ± 0.35^{ab}
Micronized to 130°C	9.17 ± 0.92^{a}	3.33 ± 0.80^{ab}
Micronized to 150°C	8.67 ± 1.12^{a}	3.37 ± 0.51^{ab}

[a-b] Means within the same column with the same letter are not significantly different (p<0.05)

The raw no-binder bologna batter was found to have significantly lower viscosity ($p<0.05$) than the batters that contained binders. According to Claus et al. (1990), Claus & Hunt (1991), and Aberle et al. (2001), viscosity of the comminuted meat batter in the absence of any plant based extender may decrease as more water is added or as fat content is lowered. Binders, mostly plant substances, are high in protein and carbohydrates content which may serve as water and fat binding agents leading to an increase in batter viscosity (Heinz & Hautzinger, 2007). No significant ($p>0.05$) difference of batter viscosity was found between batters prepared with wheat flour or chickpea flours (Table 4.2). Moreover, there were no significant ($p<0.05$) effect of seed tempering moisture conditions and micronization temperatures on the viscosity of the meat batters. This is interesting as in the previous chapter, chickpea flour from 22% initial seed moisture had higher water holding capacity than chickpea flour from seed tempered to 15% and untempered chickpea seed at corresponding temperatures, suggesting there may not be a direct link.

Substitution of meat with binders increased viscosity of batters by 20-50%. Sanjeewa et al. (2010) found the addition of 5% level of chickpea flour, Kabuli (22% protein) and Desi (23% protein), wheat flour (14.1% protein) and pea flour (15.45% protein) increased batter viscosity by 38%, 45%, 55% and 49%, respectively, compared to the control (no binder). Shand (2000) observed that batter viscosity increases of 32% and 35% were found in the bologna with addition of 4% level of wheat flour (13.4% protein) and normal barley flour (11.8% protein), respectively, relative to the control, which were similar magnitude to the present study.

(b) Proximate composition and pH of cooked bologna

The proximate composition of low-fat pork bologna products with and without binders is shown in Table 4.3. The pH of low-fat pork bologna ranged from 6.25 to 6.36, while that of the meat alone was 5.73. This increase in pH value for the bologna is expected since all material used influenced the pH value of final cooked product (Puolanne et al., 2001). In this study, all treatments contained 0.20% sodium tripolyphosphate (pH~ 9.20-10.20) which would have contributed to the increased pH.

Table 4.3 Proximate composition of cooked bologna formulated with different chickpea flours. Data represent the mean ± one standard deviation (n = 3).

Treatment	Moisture (%)	Protein (%)	Fat (%)^{ns}	Ash (%)	pH
No binder	74.56 ± 1.37^{a}	11.45 ± 0.27^{ab}	10.25 ± 0.45	2.67 ± 0.01^{bc}	6.31 ± 0.01^{abc}
Wheat flour	71.11 ± 1.87^{b}	11.12 ± 0.22^{c}	10.12 ± 0.99	2.66 ± 0.05^{c}	6.30 ± 0.03^{abc}
Chickpea flour					
Untempered					
Non-micronized	70.64 ± 1.52^{b}	11.50 ± 0.17^{ab}	10.67 ± 0.58	2.80 ± 0.04^{a}	6.36 ± 0.05^{a}
Micronized to 115^{o}C	70.75 ± 1.57^{b}	11.49 ± 0.13^{ab}	10.64 ± 0.81	2.74 ± 0.06^{ab}	6.33 ± 0.01^{ab}
Micronized to 130^{o}C	70.73 ± 1.66^{b}	11.59 ± 0.13^{a}	10.10 ± 1.58	2.80 ± 0.05^{a}	6.30 ± 0.00^{abc}
Micronized to 150^{o}C	70.89 ± 1.06^{b}	11.52 ± 0.19^{ab}	10.12 ± 0.79	2.76 ± 0.02^{a}	6.32 ± 0.07^{ab}
Tempered to 15% seed moisture					
Micronized to 115^{o}C	70.75 ± 1.58^{b}	11.29 ± 0.26^{bc}	10.89 ± 0.90	2.77 ± 0.03^{a}	6.31 ± 0.01^{abc}
Micronized to 130^{o}C	70.65 ± 1.70^{b}	11.53 ± 0.20^{ab}	10.34 ± 0.48	2.80 ± 0.03^{a}	6.27 ± 0.03^{bc}
Micronized to 150^{o}C	70.84 ± 1.61^{b}	11.51 ± 0.17^{ab}	10.48 ± 0.61	2.78 ± 0.02^{a}	6.32 ± 0.04^{ab}
Tempered to 22% seed moisture					
Micronized to 115^{o}C	70.82 ± 1.67^{b}	11.57 ± 0.19^{ab}	10.80 ± 0.51	2.79 ± 0.01^{a}	6.32 ± 0.03^{ab}
Micronized to 130^{o}C	70.89 ± 1.45^{b}	11.53 ± 0.23^{ab}	10.69 ± 0.50	2.76 ± 0.00^{a}	6.27 ± 0.02^{bc}
Micronized to 150^{o}C	70.77 ± 1.50^{b}	11.46 ± 0.19^{ab}	10.60 ± 1.16	2.75 ± 0.00^{ab}	6.25 ± 0.04^{c}

$^{a-c}$ Means within the same column with the same letter are not significantly different (p<0.05)

The bologna composition ranged from 70.64 to 74.56% moisture, 11.12 to 11.59% protein, 10.12 to 10.89% fat and 2.66 to 2.80% ash. The low-fat pork bologna product with any added binder was found to contain significantly (p<0.05) less moisture (70.64-71.11%) than the control (without binder, 74.56%). This is presumed to be due to the higher amount of pork shoulder picnic and pork backfat (containing moisture) which was added into the control (Table 4.1) to meet the target fat and protein of the final product of 10% and 11%, respectively. However no difference (p>0.05) in moisture levels were observed between the binder type (wheat vs. chickpea) or among chickpea flour treatments from tempered and micronized seed.

Protein content of low-fat pork bologna ranged from 11.12 to 11.59%, which meets the Canadian standards for protein content in ready-to-eat sausage (i.e., minimum of 9.5% derived from meat protein and 11% total protein). Bologna prepared with wheat flour had significantly (p<0.05) lower protein content (11.12%) than all samples containing chickpea flour (11.28-11.59%). This may be because wheat flour itself had lower protein content (12.60%) than chickpea flour (19.92%). There was no significant (p<0.05) difference found in terms of the fat content between samples, giving an average amount of 10.48 ± 0.28%. The low-fat pork bologna with chickpea flour was found to be significantly (p<0.05) higher in ash content (2.75-2.80%) than that with wheat flour (2.66%), however the ash content of bologna containing wheat flour was not significantly (p<0.05) different from the control (no binder). This may be due to the lower ash content of wheat flour itself (1.16%) compared to chickpea flours (2.74-2.87%).

(c) Cook loss, expressible moisture and purge losses

Table 4.4 shows the effect of flour binders on cook loss, expressible moisture and purge losses of cooked low-fat pork bologna.

Table 4.4 Effect of seed tempering moisture and micronization temperature of chickpea flour on cook loss, purge losses and expressible moisture of cooked low-fat pork bologna. Data represent the mean ± one standard deviation (n = 3).

Treatments	Cook loss (%)	Expressible moisture (%)	Purge losses (%)
No binder	0.33 ± 0.05^{ab}	18.52 ± 2.20^{a}	5.40 ± 0.27^{a}
Wheat flour	0.28 ± 0.03^{b}	9.97 ± 1.56^{c}	3.17 ± 0.48^{d}
Chickpea flour			
Untempered			
Non-micronized	0.42 ± 0.05^{a}	15.30 ± 1.83^{b}	3.86 ± 0.67^{cd}
Micronized to 115°C	0.43 ± 0.07^{a}	14.56 ± 2.15^{b}	4.36 ± 0.42^{bc}
Micronized to 130°C	0.35 ± 0.05^{ab}	14.45 ± 1.88^{b}	4.62 ± 0.60^{abc}
Micronized to 150°C	0.33 ± 0.06^{ab}	14.10 ± 1.70^{b}	4.36 ± 0.25^{bc}
Tempered to 15% seed moisture			
Micronized to 115°C	0.35 ± 0.03^{ab}	14.45 ± 1.91^{b}	4.53 ± 0.42^{bc}
Micronized to 130°C	0.40 ± 0.11^{ab}	14.29 ± 0.25^{b}	4.01 ± 0.51^{c}
Micronized to 150°C	0.38 ± 0.06^{ab}	14.77 ± 1.72^{b}	4.30 ± 0.34^{bc}
Tempered to 22% seed moisture			
Micronized to 115°C	0.32 ± 0.03^{ab}	14.83 ± 1.76^{b}	4.39 ± 0.34^{bc}
Micronized to 130°C	0.34 ± 0.06^{ab}	14.80 ± 2.01^{b}	4.26 ± 0.21^{bc}
Micronized to 150°C	0.38 ± 0.08^{ab}	15.03 ± 2.06^{b}	4.81 ± 0.42^{ab}

$^{a\text{-}d}$ Means within the same column with the same letter are not significantly different ($p<0.05$)

Cook loss refers to the quantity of moisture or weight loss from the product after the cooking process. Measuring cook loss is important for the industry as an economic reason since it has an influence on product yield, and it is also important for the consumer in term of eating quality. The low-fat pork bologna that contained wheat flour as a binder had the lowest cook loss (0.28%) whereas, the bologna with chickpea flour from non-micronized seed or that from seed micronized to 115°C without tempering had the highest cook loss (0.42-0.43%). The lowest cook loss of bologna produced with wheat flour may be due to the high carbohydrate content of flour itself (~72%) compared to chickpea flour (~40%). Heinz & Hautzinger (2007) stated that the high-carbohydrate substances could provide significantly volume increase, as it is highly water absorbent. There was no significant (p>0.05) difference in cook loss among the control (no binder) (0.33%) and all chickpea flour treatments (0.32 – 0.43%), showing no benefit of these binders for this property.

Expressible moisture and purge losses were determined in order to quantify the water loss of cooked bologna by a centrifugation method and a gravitational drip method, respectively. The amount of fluid lost during vacuum packaged storage (purge losses) relates to consumer acceptability and shelf life of the product. The consumer buying decision is based on appearance which is the most important product attribute that consumers use to evaluate the product quality (Resurreccion, 2003). The usefulness of having binders in the meat system was found in this study. Expressible moisture and purge losses of all bologna containing binders were significantly (p<0.05) lower compared to the control bologna (no binder) since binders are high in carbohydrates which serve as water binding agents. Expressible moisture within low-fat cooked bologna was reduced from 18.52% for products without binder to 14.10-15.30% and 9.97% in bologna with chickpea and wheat flour respectively (p<0.05). Purge loss of low-fat cooked bologna ranged from 3.17 to 5.40%. The bologna with wheat flour showed a significantly (p<0.05) lower purge loss value (3.17%) than bologna containing chickpea flour (3.86-4.81%) and the control (5.40%). No difference (p>0.05) in expressible moisture was seen among the various chickpea flour treatments, showing no benefit of seed tempering moisture and micronization temperature on these attributes. This study showed bologna produced with wheat flour, the industry standard for binders in bologna, had higher water holding capacity than chickpea based bologna.

Sanjeewa (2008) reported when adding 5% (w/w) wheat flour into low-fat pork bologna, the lowest expressible moisture (9.55%) was found. In addition, the expressible moisture of bologna with added wheat flour was less than bologna with 5% Kabuli chickpea flour from non-micronized seed and the control, 10.28 and 17.33%, respectively. Lower purge losses with addition of a binder was observed whereas there was no significant (p<0.05) difference between bologna added wheat flour and chickpea flour from non-micronized seed, showing that chickpea flour was equally as effective as wheat flour in that study. Moreover, several studies have shown that beneficial effects of binders to increase water holding capacity of meat products compared to those without binders. For example, Shand (2000) reported purge loss and expressible moisture of low-fat pork bologna containing binders were significantly lower than the no binder treatment. Brown & Zayas (1990) showed extended beef patties (contained 10-30% corn germ protein flour) had lower cooking losses than control patties. Dzudie et al. (2002) concluded that WHC and cooking losses of beef sausages decreased with increasing level of added common bean flour. In addition, Serdaroğlu et al. (2005) found that meatballs produced with legume (including blackeye bean, chickpea, lentil) flours at level of 10% had higher cook yield than meatballs extended with rusk, which usually used as a binder or extender in meatballs.

(d) CIE colour

Meat colour is an important characteristic associated with consumer preferences. The red pigment in muscle (myoglobin) which becomes nitrosylhemochrome on curing is usually the dominant colour of processed meats, but does become more pale with addition of fat. The colour of final products may be influenced by other ingredients when added to processed meats. Data for colour of the control bologna and bologna containing various binders are presented in Table 4.5.

The L* value of the bologna, which indicates the lightness, ranged from 73.36 to 75.77. No significant (p>0.05) difference of L* value or lightness was found between bologna formulations with wheat flour (74.38) and no binder (75.01). Type of binders (wheat flour or chickpea flour) addition did not affect L* value of cooked samples. There was no clear effect of micronized temperature and initial seed moisture on L* value of low-fat pork bologna. Although chickpea flour from untempered seed was significantly (p>0.05) lighter in colour than that from seed tempered to 15% and 22% seed moisture and micronized to 150°C, the colour difference was not found when these flours were incorporated into low-fat pork bologna. However, the highest L* value was observed when adding chickpea flour from seed tempered to 15% moisture and micronized to 115°C (75.77). Adding chickpea flour from seed tempered to 22% seed moisture with micronization to 150°C provided bologna with the lowest L* value (73.36).

Table 4.5 Effect of seed tempering moisture and micronization temperature of chickpea flour on bologna colour. Data represent the mean ± one standard deviation (n = 3).

Treatment	CIE colour[1]		
	L*	a*	b*
No binder	75.01 ± 1.52^{abc}	16.73 ± 0.88^{a}	16.23 ± 0.39^{d}
Wheat flour	74.38 ± 1.57^{abc}	16.82 ± 0.69^{a}	18.23 ± 0.12^{c}
Chickpea flour			
Untempered			
Non-micronized	73.95 ± 0.92^{bc}	15.88 ± 0.88^{bcd}	21.91 ± 0.27^{a}
Micronized to 115^{o}C	74.50 ± 0.67^{abc}	15.82 ± 0.93^{cd}	20.83 ± 0.49^{b}
Micronized to 130^{o}C	75.26 ± 1.66^{ab}	15.84 ± 1.11^{bcd}	21.11 ± 0.26^{ab}
Micronized to 150^{o}C	74.51 ± 1.46^{abc}	16.31 ± 1.03^{abc}	21.50 ± 0.21^{ab}
Tempered to 15% seed moisture			
Micronized to 115^{o}C	75.77 ± 0.51^{a}	15.27 ± 0.62^{d}	20.95 ± 0.25^{b}
Micronized to 130^{o}C	74.61 ± 1.77^{abc}	16.61 ± 1.10^{ab}	21.41 ± 0.44^{ab}
Micronized to 150^{o}C	74.64 ± 1.60^{abc}	16.35 ± 0.97^{abc}	21.44 ± 0.45^{ab}
Tempered to 22% seed moisture			
Micronized to 115^{o}C	74.84 ± 1.90^{abc}	16.22 ± 1.13^{abc}	21.05 ± 0.39^{ab}
Micronized to 130^{o}C	74.67 ± 1.26^{abc}	16.14 ± 0.99^{abc}	20.89 ± 0.10^{b}
Micronized to 150^{o}C	73.36 ± 1.17^{c}	15.61 ± 0.93^{cd}	21.53 ± 0.32^{ab}

$^{a-d}$ Means within the same column with the same letter are not significantly different (p<0.05)
[1]CIE colour: "L*" = lightness; "a*" = redness; "b" = yellowness

A similar trend was observed for a* (redness) values of bologna, where wheat flour addition had no effect on redness. Moreover, the control bologna and that with wheat flour had higher a* values (16.13 and 16.82, respectively) than that of bologna contained chickpea flour (a* values ranged from 15.27 to 16.61). The a* value of the control bologna (16.73) and for those products with wheat flour (16.82) were found to be significantly (p<0.05) different from bologna contained chickpea flour from non-micronized seed (15.88), untempered chickpea seed following by micronization to 115°C (15.82) and 130°C (15.84), 15% seed moisture with 115°C of micronization (15.27) and 22% seed moisture with 150°C of micronization (15.61).

Bologna formulations with wheat flour had significantly (p<0.05) higher CIE yellowness (b*) compared with the control (18.23 versus 16.23). The highest b* value was observed for low-fat pork bologna containing chickpea flour from non-micronized seed (21.91). The addition of chickpea flour significantly (p<0.05) increased the yellowness (b*) of the bologna, with values ranging from 20.83 to 21.91, compared with the control (16.23) and wheat flour (18.23), whereas, there was no significant (p<0.05) difference in b* value among all chickpea flour from micronized seed added bologna samples. The minor differences among chickpea flour added bologna might be due to the changes in colour of the chickpea seed coat which was affected by the level of initial seed moisture before micronization and micronization temperature.

In general, addition of wheat flour did not affect lightness (L*) and redness (a*) of bologna compared to the control. The addition of chickpea flour increased yellowness (b*), compared to bologna produced with wheat flour and the control. This study showed no benefit of seed tempering moisture and micronization temperature on the colour of bologna with added chickpea flour, but also only minor negative impact. Similar findings were reported by Sanjeewa (2008) who found that addition of non-micronized chickpea flour at the level of 5% into low-fat pork bologna showed no significant effect (p>0.05) on L* and a* values relative to bologna containing wheat flour and the control. Chickpea flour from non-micronized seed added to bologna was significantly (p<0.05) more yellow than bologna containing wheat flour and the control, as was shown in the present study. Dzudie et al. (2002) stated that beef sausages extended with at the level of 7.5% and 10% chickpea flour were more yellow than the sample without added chickpea flour. Shand (2000) reported that addition of wheat flour and barley flour at the level of 4% had minor effects on CIE colour (L*, a*, b*) of ultra low-fat (<1%) pork bologna.

(e) Instrumental texture

Texture profile analysis results for the low-fat pork bologna with the various binders are shown in Table 4.6.

Table 4.6 Effect of different flour binders on textural properties of cooked low-fat bologna. Data represent the mean ± one standard deviation (n = 3).

Treatment	Texture profile analysis				
	Hardness (N)	Adhesiveness (N)	Cohesiveness	Springiness (%)	Chewiness (N mm)
No binder	61.98 ± 2.21^{d}	-0.81 ± 0.07^{c}	0.57 ± 0.01^{a}	80.37 ± 2.72^{ab}	356.16 ± 11.90^{f}
Wheat flour	82.49 ± 8.20^{ab}	-1.18 ± 0.07^{a}	0.56 ± 0.00^{ab}	82.58 ± 1.79^{a}	487.05 ± 38.42^{a}
Chickpea flour					
Untempered					
Non-micronized	69.56 ± 2.18^{cd}	-1.08 ± 0.15^{ab}	0.54 ± 0.01^{b}	78.92 ± 1.55^{b}	377.81 ± 11.73^{ef}
Micronized to 115^{o}C	68.65 ± 5.50^{cd}	-0.94 ± 0.04^{bc}	0.54 ± 0.00^{b}	78.20 ± 1.11^{b}	365.48 ± 25.03^{f}
Micronized to 130^{o}C	74.14 ± 7.44^{abcd}	-0.91 ± 0.08^{bc}	0.55 ± 0.02^{ab}	78.67 ± 1.30^{b}	403.51 ± 48.68^{bcdef}
Micronized to 150^{o}C	72.27 ± 4.65^{abcd}	-1.00 ± 0.02^{abc}	0.56 ± 0.02^{ab}	80.06 ± 0.91^{ab}	402.62 ± 22.42^{cdef}
Tempered to 15% seed moisture					
Micronized to 115^{o}C	70.54 ± 6.25^{bcd}	-0.94 ± 0.04^{bc}	0.56 ± 0.01^{ab}	78.55 ± 1.19^{b}	381.76 ± 31.79^{def}
Micronized to 130^{o}C	73.62 ± 7.22^{abcd}	-1.01 ± 0.06^{abc}	0.56 ± 0.00^{ab}	79.74 ± 0.81^{b}	410.07 ± 35.08^{bcdef}
Micronized to 150^{o}C	77.48 ± 2.31^{abc}	-0.87 ± 0.07^{c}	0.56 ± 0.02^{ab}	80.70 ± 0.91^{ab}	450.89 ± 29.11^{abc}
Tempered to 22% seed moisture					
Micronized to 115^{o}C	83.42 ± 6.04^{a}	-0.98 ± 0.06^{abc}	0.55 ± 0.01^{ab}	80.33 ± 1.48^{ab}	470.15 ± 41.31^{ab}
Micronized to 130^{o}C	79.16 ± 6.97^{abc}	-0.89 ± 0.06^{bc}	0.56 ± 0.01^{ab}	79.67 ± 1.14^{b}	446.99 ± 39.80^{abcd}
Micronized to 150^{o}C	79.29 ± 9.04^{abc}	-0.93 ± 0.07^{bc}	0.55 ± 0.01^{ab}	79.96 ± 1.78^{b}	443.00 ± 46.89^{abcde}

$^{a-f}$ Means within the same column with the same letter are not significantly different (p<0.05)

In general, type of binders added in meat products influenced the textural attributes, especially hardness. Bologna without binders was found to have significantly (p<0.05) lower hardness (61.98 N) than bologna with added wheat flour (82.49 N). There was no significant (p<0.05) difference in hardness among bologna containing wheat flour, chickpea flour from untempered seed prior micronized to 130 and 150^{o}C, chickpea flour from seed tempered to 15% and 22% moisture following 115, 130, 150^{o}C micronization, showing that these binders can be used as an alternative for maintaining hardness. However, the addition of chickpea flour from non-micronized seed and 115^{o}C micronized without tempering resulted in significantly (p<0.05) lower hardness compared to bologna produced with wheat flour.

For adhesiveness, bologna containing wheat flour had the lowest adhesiveness (-1.18 N), whereas the control showed the highest adhesiveness (-0.81 N) when compared to all other treatments. No significant change in adhesiveness was found among all bologna with addition of chickpea flour from micronized seed, moreover, these bologna were not significantly (p<0.05) different from the control. Among bologna with added chickpea flour, the lowest adhesiveness value was observed in bologna containing chickpea flour from non-micronized seed (-1.08N) and the highest value was found in bologna produced with chickpea flour from seed tempered to 15% following by 150^{o}C micronization (-0.87 N).

The addition of wheat flour did not affect (p<0.05) cohesiveness and springiness of bologna, compared with the control. Effects of binder types (wheat flour or chickpea flour), or seed tempering moisture and micronizing conditions on cohesiveness were not found in this study. The springiness of bologna prepared with wheat flour was found to be significantly (p<0.05) higher than bologna with chickpea flour from non-micronized seed, untempered seed and several of the micronization treatments (e.g., those tempered to 15% seed moisture and micronized to 115 and 130^{o}C and those tempered to 22% seed moisture following by 130 and 150^{o}C micronization).

In term of chewiness, the control (no binder) had the lowest chewiness (356.16 N mm), which was significantly (p<0.05) lower than bologna prepared with wheat flour (487.05 N mm). This study showed that chewiness of bologna containing chickpea flour from non-micronized seed, untempered seed following by 115, 130, 150^{o}C micronization, and seed tempered to 15% with micronization to reach 130^{o}C were similar to the no binder treatment. The chewiness of bologna produced with chickpea flour from seed micronized at 150^{o}C after tempered to 15%

seed moisture and all chickpea flour from seed tempered to 22% following by micronization (115, 130, 150^{o}C) was similar to bologna with added wheat flour, showing the benefit of these binders as an alternative for achieving the same chewiness.

Sanjeewa (2010) compared the TPA parameters of low-fat pork bologna with addition of 5% wheat flour and 5% chickpea flour from non-micronized seed and found that bologna produced with chickpea flour had higher hardness, cohesiveness and chewiness than bologna containing wheat flour and the control. There was no different of springiness between bologna containing chickpea flour from non-micronized seed and wheat flour. Shand (2000) found that the use of wheat flour and barley increased the hardness of ultra low-fat (<1%) pork bologna, compared with the control. However, the results from the present study differed from Verma et al. (1984) who stated that addition 15% of chickpea flour into sausages led to softer texture compared to the control when the same level of water was added. Dzudie et al. (2002) reported that the control beef sausage had the highest hardness while hardness was lowest for beef sausage containing 5.0%, 7.5% and 10.0% chickpea flour, which were not significantly (p<0.05) different from each other.

The effects of different flour binders on torsion parameters of cooked low-fat bologna are depicted in Table 4.7. Bologna containing wheat flour showed a significantly (p<0.05) higher shear stress, compared to the control, whereas there was no significantly (p<0.05) difference in shear strain. The results from this study showed that shear stress and shear strain of bologna with added chickpea flour from micronized seed was not significantly (p<0.05) affected by seed tempering moisture and micronization temperature, whereas addition of chickpea flour from tempered and micronized seed improved shear strain of the bologna, compared to flour addition from non-micronized seed. Bologna with chickpea flour from non-micronized seed had the lowest shear stress and shear strain among all bologna samples, 32.42 kPa and 1.25, respectively, whereas bologna produced with wheat flour had the highest scores, 46.79 kPa and 1.62 kPa, respectively.

Table 4.7 Effect of different flour binders on shear stress and shear strain of cooked low-fat bologna obtained from torsion analysis. Data represent the mean ± one standard deviation (n = 3).

Treatment	Torsion shear values	
	Shear stress (kPa)	Shear strain
No binder	33.38 ± 5.70^{bc}	1.61 ± 0.01^{a}
Wheat flour	46.79 ± 6.58^{a}	1.62 ± 0.14^{a}
Chickpea flour		
Untempered		
Non-micronized	32.42 ± 2.94^{c}	1.25 ± 0.10^{b}
Micronized to 115^{o}C	35.19 ± 1.70^{abc}	1.53 ± 0.07^{a}
Micronized to 130^{o}C	37.57 ± 3.75^{abc}	1.45 ± 0.08^{ab}
Micronized to 150^{o}C	37.10 ± 4.76^{abc}	1.55 ± 0.07^{a}
Tempered to 15% seed moisture		
Micronized to 115^{o}C	37.68 ± 6.39^{abc}	1.56 ± 0.10^{a}
Micronized to 130^{o}C	42.06 ± 2.40^{abc}	1.51 ± 0.09^{a}
Micronized to 150^{o}C	38.96 ± 2.38^{abc}	1.52 ± 0.07^{a}
Tempered to 22% seed moisture		
Micronized to 115^{o}C	40.62 ± 6.19^{abc}	1.55 ± 0.05^{a}
Micronized to 130^{o}C	45.00 ± 3.31^{ab}	1.56 ± 0.09^{a}
Micronized to 150^{o}C	47.02 ± 6.55^{a}	1.59 ± 0.09^{a}

$^{a-c}$ Means within the same column with the same letter are not significantly different (p<0.05)

Chang & Carpenter (1997) reported that frankfurters containing 2%, 4%, and 6% oat bran had higher shear stress and also higher shear strain than no binder treatment. Sanjeewa (2008) stated that the differences in shear stress and shear strain values of low-fat pork bologna due to type of flour added and the addition of binder increased shear stress but decreased shear strain, compared to the control. Sanjeewa (2008) found that bologna produced with 5% chickpea flour from non-micronized seed had similar shear stress with bologna prepared with 5% wheat flour whereas, in this study bologna that contained wheat flour had significantly (p<0.05) higher shear stress than control bologna with added wheat flour. Moreover, the control bologna was found in Sanjeewa (2008) to have lower shear stress than bologna produced with 5% chickpea flour from non-micronized seed while, there was no significantly (p<0.05) difference between those two treatments in shear stress found in this study. However, corresponded results were found between this study and Sanjeewa (2008) that bologna containing 5% chickpea flour from non-micronized seed showed the lower shear strain than bologna produced with wheat flour, nevertheless, the lower shear strain of bologna with added wheat flour compared to control from Sanjeewa (2008) was not found in this study. The most probable reason could not be explained but can be related to the high standard deviation of the shear strain for bologna added with wheat flour (Table 4.7, 1.62 ± 0.14).

(f) Sensory evaluation

For determining the sensory attributes, twelve treatments were evaluated by twelve trained sensory panelists. The average results for the sensory evaluation of low-fat pork bologna are presented in Table 4.8. Bologna colour was influenced significantly (p<0.05) by the addition of chickpea flour sample relative to wheat flour and/or no binder present. Bologna without binders had significantly (p<0.05) higher redness score (6.08) than bologna produced with wheat flour (5.42), and chickpea flour (3.72-4.11), respectively. Different micronization and seed tempering moisture condition of chickpea seed did not affect the colour of bologna with added chickpea flour (p<0.05), showing no benefit of seed tempering moisture and micronization temperature on bologna colour. The sensory results were similar to the colour results obtained from the CIE Lab system as the results showed that bologna contained chickpea flour was significantly (p<0.05) higher in yellow colour (b* value), compared to those with wheat flour and the no binder treatment.

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Table 4.8 Effect of different flour binders on sensory parameters of cooked low fat bologna. Data represent the mean ± one standard deviation (n = 3).

Treatment	Sensory parameters				
	Colour	Firmness	Chewiness	Juiciness	Saltiness
No binder	6.08 ± 0.14^{a}	4.00 ± 0.30^{cd}	3.42 ± 0.52^{bc}	5.69 ± 0.17^{a}	3.19 ± 0.95^{bc}
Wheat flour	5.42 ± 0.14^{b}	5.36 ± 0.34^{a}	4.31 ± 0.38^{a}	4.67 ± 0.36^{b}	3.03 ± 0.97^{c}
Chickpea flour					
Untempered					
Non-micronized	3.75 ± 0.14^{c}	3.14 ± 0.38^{e}	2.83 ± 0.33^{c}	4.50 ± 0.36^{b}	3.72 ± 0.74^{a}
Micronized to 115°C	4.06 ± 0.17^{c}	3.56 ± 0.38^{de}	2.97 ± 0.34^{c}	4.75 ± 0.17^{b}	3.56 ± 0.81^{ab}
Micronized to 130°C	3.89 ± 0.13^{c}	4.17 ± 0.82^{bcd}	3.39 ± 0.72^{bc}	4.72 ± 0.17^{b}	3.42 ± 0.84^{abc}
Micronized to 150°C	4.08 ± 0.08^{c}	4.22 ± 0.29^{bcd}	3.50 ± 0.29^{bc}	4.81 ± 0.05^{b}	3.50 ± 0.65^{abc}
Tempered to 15% seed moisture					
Micronized to 115°C	3.81 ± 0.05^{c}	4.11 ± 0.83^{cd}	3.50 ± 0.44^{bc}	4.78 ± 0.10^{b}	3.50 ± 0.94^{abc}
Micronized to 130°C	4.03 ± 0.41^{c}	4.17 ± 0.55^{bcd}	3.50 ± 0.38^{bc}	4.83 ± 0.33^{b}	3.42 ± 0.87^{abc}
Micronized to 150°C	3.72 ± 0.34^{c}	4.50 ± 0.22^{bc}	3.69 ± 0.35^{ab}	4.53 ± 0.17^{b}	3.50 ± 0.81^{abc}
Tempered to 22% seed moisture					
Micronized to 115°C	3.94 ± 0.10^{c}	4.53 ± 0.73^{bc}	3.69 ± 0.65^{ab}	4.50 ± 0.22^{b}	3.39 ± 0.84^{abc}
Micronized to 130°C	4.11 ± 0.17^{c}	4.56 ± 0.39^{bc}	3.83 ± 0.30^{ab}	4.75 ± 0.14^{b}	3.50 ± 0.74^{abc}
Micronized to 150°C	3.78 ± 0.10^{c}	4.83 ± 0.51^{ab}	3.94 ± 0.41^{ab}	4.81 ± 0.17^{b}	3.36 ± 0.80^{abc}

$^{a-f}$ Means within the same column with the same letter are not significantly different (p<0.05)

Colour : 8 = Extremely pinkish red, 1 = Extremely brown; Firmness: 8 = Extremely firm, 1 = Extremely soft; Chewiness: 8 = Extremely hard to chew, 1 = Extremely easy to chew; Juiciness: 8 = Extremely juicy, 1 = Extremely dry; Saltiness: 6 = Extremely salty, 1 = not detectable

Table 4.8 Continued…

Treatment	Sensory parameters			
	Flavour intensity	Flavour desirability	Foreign flavour	Overall acceptability
No binder	4.89 ± 0.17[bc]	5.44 ± 0.29[ab]	2.56 ± 0.24[f]	5.47 ± 0.55[a]
Wheat flour	4.44 ± 0.49[c]	4.61 ± 0.13[cde]	2.92 ± 0.36[ef]	4.56 ± 0.10[bc]
Chickpea flour				
Untempered				
Non-micronized	5.50 ± 0.08[ab]	4.33 ± 0.33[e]	4.86 ± 0.29[a]	4.28 ± 0.49[c]
Micronized to 115°C	5.69 ± 0.21[a]	4.50 ± 0.44[de]	4.67 ± 0.36[ab]	4.58 ± 0.25[bc]
Micronized to 130°C	5.42 ± 0.25[ab]	4.64 ± 0.38[bcde]	4.31 ± 0.13[abc]	4.61 ± 0.34[bc]
Micronized to 150°C	5.69 ± 0.21[a]	5.44 ± 0.42[ab]	3.22 ± 0.96[def]	5.47 ± 0.29[a]
Tempered to 15% seed moisture				
Micronized to 115°C	5.75 ± 0.66[a]	5.19 ± 0.46[abcd]	4.22 ± 0.87[abcd]	5.17 ± 0.52[ab]
Micronized to 130°C	5.56 ± 0.24[ab]	5.03 ± 0.46[abcde]	3.56 ± 0.49[cdef]	5.06 ± 0.51[abc]
Micronized to 150°C	5.81 ± 0.17[a]	4.78 ± 0.21[abcde]	4.25 ± 0.22[abcd]	4.69 ± 0.13[abc]
Tempered to 22% seed moisture				
Micronized to 115°C	5.36 ± 0.39[ab]	5.47 ± 0.05[a]	3.67 ± 0.38[bcde]	5.39 ± 0.13[ab]
Micronized to 130°C	5.56 ± 0.29[ab]	5.33 ± 0.30[abc]	3.89 ± 0.25[abcde]	5.36 ± 0.13[ab]
Micronized to 150°C	6.03 ± 0.13[a]	5.03 ± 0.46[abcde]	4.58 ± 0.22[abc]	4.89 ± 0.41[abc]

[a-f] Means within the same column with the same letter are not significantly different (p<0.05)
Flavour intensity: 8 = Extremely intense, 1 = Extremely bland
Flavour desirability: 8 = Extremely desirable, 1 = Extremely undesirable
Foreign flavour: 8 = Extremely intense, 1 = No foreign flavour
Overall acceptability: 8 = Extremely acceptable, 1 = Extremely unacceptable

The sensory panel results for firmness and chewiness were consistent with the texture profile analysis (TPA) data. Bologna containing wheat flour had significantly (p<0.05) higher firmness (5.36) and chewiness (4.31) scores than the control (4.00 and 3.41, respectively), while bologna with chickpea flour from non-micronized seed had the lowest values (3.14 and 2.83, respectively). There was no significant (p<0.05) difference in firmness between bologna containing chickpea flour from non-micronized seed and chickpea flour from seed micronized at 115^{o}C without tempering, whereas bologna containing other chickpea flour treatments had significantly (p<0.05) higher firmness scores than bologna produced with chickpea flour from non-micronized seed. Bologna with chickpea flour from seed tempered to 22% seed moisture following 150^{o}C micronization showed similar firmness to wheat flour, whereas bologna containing other chickpea flour were significantly (p<0.05) lower in firmness score. These data showed the benefit of micronization on firmness of bologna.

The chewiness score of bologna with added chickpea flour from seed tempered to 15% moisture following by 150^{o}C micronization and chickpea flour from seed tempered to 22% following by micronization (115, 130, 150^{o}C) were not significantly (p<0.05) different than bologna with added wheat flour. These results were similar to the instrumental texture results obtained from TPA. Moreover, there was a difference in sensory chewiness due to the effect of seed tempering moisture when followed by low micronized temperature. When adding chickpea flour from seed micronized to 115^{o}C at 22% initial seed moisture into low-fat pork bologna, firmness and chewiness increased significantly (p<0.05) comparing to those with added chickpea flour from untempered seed at corresponding micronized temperature. This likely was due to the effect of gelatinized starch.

For juiciness, the control (no binder) bologna had the greatest juiciness among panelists, and was significantly (p<0.05) juicier than samples with wheat flour and all chickpea flours. However, juiciness was not affected by type of binders in this study. Moreover, there was no significant (p<0.05) effect of seed tempering moisture and micronization conditions on juiciness.

This study showed the potential of addition of chickpea flour into low-fat pork bologna by not changing juiciness (compared to wheat flour), so it can be used as an alternative binder and this property would be maintained. These sensory results corresponded to the proximate (Table 4.3) and water binding capacity (Table 4.4) of bologna. Since the no binder bologna contained highest amount of moisture among all bologna with the addition of binders, and it had highest expressible moisture and purge losses, these factors influenced the characteristics of final product by showing an increase in juiciness perception.

No significant (p<0.05) differences in saltiness intensity were found between the control (no binder) and bologna produced with wheat flour. While the saltiness of the bologna with chickpea flour from non-micronized seed was the highest, and significantly higher than the bologna with wheat flour, it did not differ significantly from any of the other bologna with added chickpea flours. Saltiness was not significantly (p<0.05) changed among bologna with added chickpea flour, showing that seed tempering moisture and micronization conditions did not affect the perception of this property. Moreover, the lack of difference in saltiness between chickpea samples in this study might due to the masking of saltiness by the flavour of chickpea flour.

Flavour is one of the most important factors in quality and acceptability of food products, which is perceived through interaction of the sense of taste and odor compounds. Matrix structure, chemical and physical properties of foods influence the release of compounds from the food matrix. The intensity of volatile and non-volatile flavour compounds released have an effect on flavour perception (Ross, 2009). The flavour intensity of bologna produced with wheat flour (4.44) was not significantly (p<0.05) different from the control (4.89). However, bologna containing wheat flour had significantly (p<0.05) lower flavour intensity score than all bologna with addition of chickpea flour (5.36-6.03). This study showed that both seed tempering moisture and micronization conditions had no effect on flavour intensity since the difference of this property was not found among all bologna produced with chickpea flour. The interesting flavour intensity results of this study were not expected since Shariati-Ievari (2013) reported that the concentrations of volatile compounds were significantly (p<0.05) decreased in lentil and chickpea flour with micronization of chickpea and lentil seed.

Bologna prepared with wheat flour showed a significantly (p<0.05) lower flavour desirability scores (4.61) than the control (5.44), whereas there was no significantly (p<0.05) difference between bologna containing wheat flour and chickpea flour from non-micronized seed (4.33). The desirability of flavour was significantly (p<0.05) affected by seed tempering moisture when chickpea seed was micronized to 115^{o}C, whereas no significant (p<0.05) effect was found in chickpea flour from seed micronized to 130 and 150^{o}C at corresponding initial seed moisture. Bologna samples with chickpea flour from seed tempered to 22% seed moisture and from seed micronized to 115^{o}C had the highest flavour desirable score. In addition, it was significantly higher (p<0.05) than the bologna contained wheat flour, chickpea flour from non-micronized seed and from seed micronized at 115^{o}C and 130^{o}C without tempering. Thus micronization increased flavour desirability. It might be because micronization acted to reduce the concentrations of volatile compounds such as pentanol, hexanal, 2-hexenal, hexanol, heptanal, furan-2-pentyl, 2-octenal, nonanal, 2,4 decadienal, and 2,4- undecadienal which may be responsible for unpleasant 'beany' aroma and flavour of chickpea flour (Shariati-Ievari, 2013).

No significant (p<0.05) differences of foreign flavour score were found between the control (2.56) and bologna produced with wheat flour (2.92). Panelists scored the bologna with chickpea flours from non-micronized seed to have highest foreign flavour (4.86) among all bologna treatments showing a benefit for micronization. The overall acceptability of bologna containing wheat flour (4.56) was significantly lower (p<0.05) than the control (5.47), while there were no significant (p<0.05) differences between bologna prepared with wheat flour and chickpea flour from non-micronized seed. The effect of micronization temperatures on seed tempering moisture (15% and 22% seed moisture) was not found in this study. Panelists scored the bologna with chickpea flours from seed micronized to 150^{o}C without tempering to have highest overall acceptability (5.47) among all bologna treatments. Moreover, this treatment was not significantly (p<0.05) different from the control and bologna containing chickpea flour from micronized seed (115, 130, 150^{o}C) after tempered to 15% and 22% seed moisture, showing that these chickpea flours have a benefitial effect on overall acceptability of low-fat pork bologna.

Sanjeewa (2008) reported that there was no significant (p<0.05) difference of overall flavour intensity, flavour desirability, and foreign flavour intensity between the control (no binder) and bologna with the addition of 5% chickpea flour from non-micronized seed while the trained panel from this study indicated that bologna produced with chickpea flour from non-micronized seed had less flavour desirability and was higher in foreign flavour compared to the control.

The sensory properties of meat products with the addition of legume flour were investigated. Kurt & Kilinççeker (2012) studied and compared the sensory quality of the addition of 5% legumes flours (chickpea and lentil) into beef patties. The patties with chickpea flour had better sensory quality attributes (appearance, colour, odour, texture, flavour and overall quality) compared to patties produced with lentil flour, moreover patties containing chickpea flour was not significantly (p<0.05) different from the patties with wheat flour. Serdaroğlu et al. (2005)

found that there were no differences in appearance and flavour scores of meatball containing 10% blackeye bean flour, chickpea flour, and lentil flour. Meatball samples produced with chickpea flour had the lowest texture and overall palatability scores. Moreover, Prinyawiwatkul et al. (1997) reported addition of 10% cowpea flour decreased flavour scores of chicken nuggets. Modi et al. (2003) found the buffalo burger with 8% black gram dhal flour had better sensory quality attributes compared to other legumes (soya, bean, Bengal gram, green gram, and black gram).

Table 4.9 shows the Pearson correlation analysis of the instrumental texture data and the sensory data. Significant positive relationships were found between TPA-hardness (r = 0.75, p<0.01), TPA-springiness (r = 0.77, p<0.01), TPA-chewiness (r = 0.84, p<0.001), shear stress (r = 0.87, p<0.001), shear strain (r = 0.72, p<0.01) and sensory firmness.

Since there was a strong positive correlation between sensory firmness and sensory chewiness (r = 0.99 p<0.001), chewiness data obtained from sensory evaluation had significant positive relationship to data obtained from instrumental texture, similar to sensory firmness. Flavour intensity and foreign flavour had significantly (r = 0.74, p<0.01 and r = 0.75, p<0.01, respectively) positive relationship with the saltiness score. Interestingly, overall acceptability had a positive relationship with low foreign flavour (r = 0.59, p<0.05), while a strong positive relationship was found between overall acceptability and flavour desirability (r = 0.99 p<0.001). This indicated that the strongest driver for overall acceptability may be related to flavour.

Table 4.9 Correlation coefficients (r) among instrumental texture measurements and sensory data of low-fat pork bologna (combined data, n=36)

	8	9	10	11	12	13	14	15
Instrumental Texture Analysis								
1. TPA-Hardness	0.75**	0.72**	-0.65*	-0.24	-0.03	0.04	0.05	-0.05
2. TPA-Adhesiveness	-0.17	-0.17	0.50	0.15	0.40	0.42	0.05	0.43
3. TPA-Cohesiveness	0.46	0.53	0.59*	-0.57	-0.34	0.63*	-0.79**	0.64*
4. TPA-Springiness	0.77**	0.78**	0.05	-0.76**	-0.62*	0.16	-0.67*	0.10
5.TPA-Chewiness	0.84***	0.83***	-0.51	-0.41	-0.20	0.09	-0.15	0.00
6. Torsion-Shear stress	0.87***	0.87***	-0.25	-0.48	-0.06	0.16	-0.13	0.09
7. Torsion-Shear strain	0.72**	0.71*	0.43	-0.71**	-0.22	0.58*	-0.59*	0.58
Sensory Attributes								
8. Firmness	1	0.99***	-0.08	-0.73**	-0.31	0.29	-0.43	0.21
9. Chewiness		1	-0.04	-0.73	-0.32	0.33	-0.46	0.25
10. Juiciness			1	-0.44	-0.31	0.43	-0.59*	0.48
11. Saltiness				1	0.74**	-0.22	0.75**	-0.20
12. Flavour intensity					1	0.06	-0.70*	0.03
13. Flavour desirability						1	-0.56	0.99***
14. Foreign flavour							1	0.59*
15. Overall acceptability								1

*, **, *** = Significant at p<0.05, 0.01 and 0.001, respectively

These sensory correlations corresponded with the prediction obtained from contour plots as shown below. Multiple regression models, control plots, were determined from sensory data to develop optimum levels of micronization temperature and seed tempering moisture conditions of chickpea seed prior milled to yield a flour following its incorporation into the model low-fat pork bologna product (Figure 4.1, Figure 4.2 and Figure 4.3).

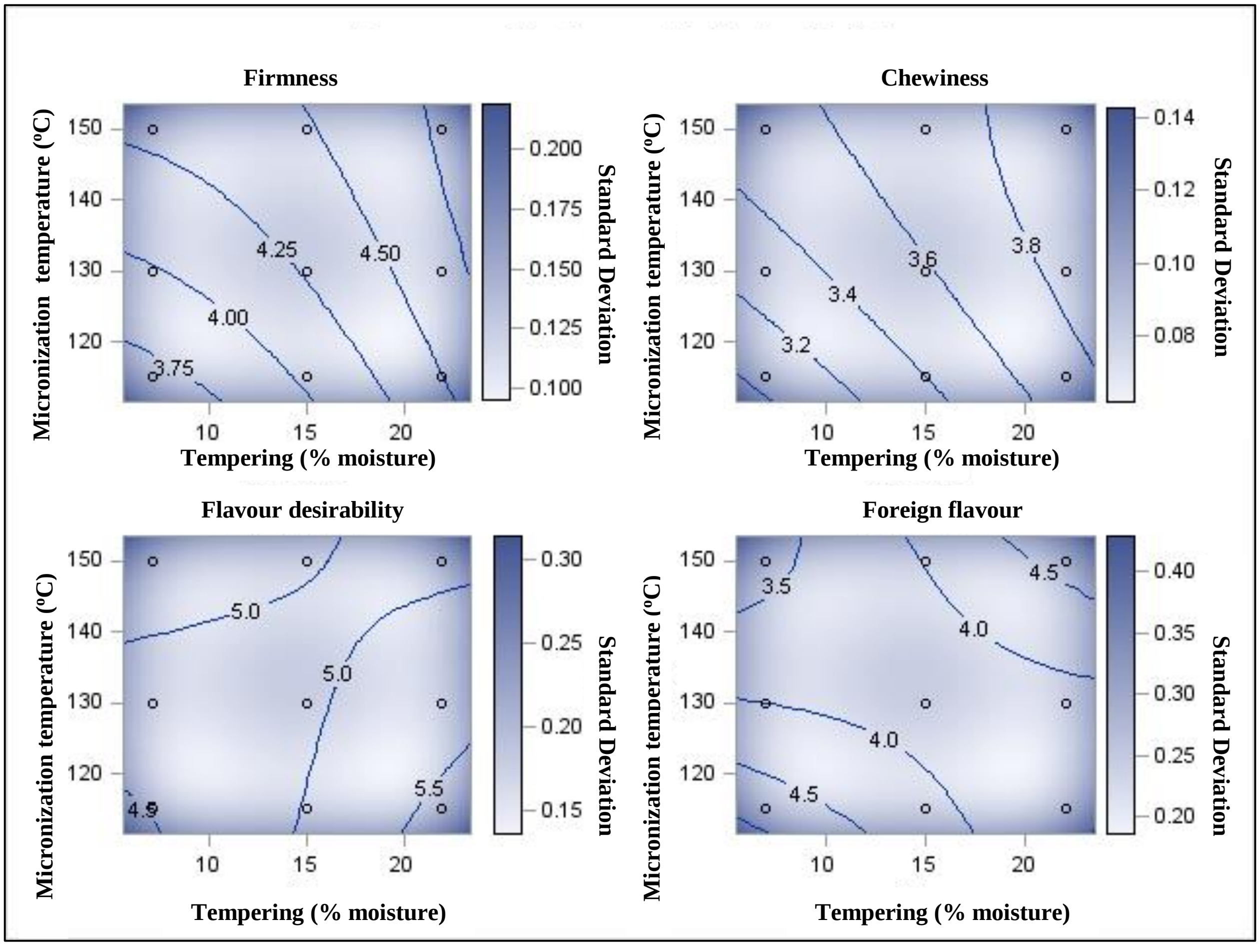

Figure 4.1 Contour plot for sensory firmness, chewiness, flavour desirability and foreign flavour (n = 9). Firmness: 5 = Slightly firm, 4 = Slightly soft and 3 = Moderately soft; Chewiness: 4 = Slightly easy to chew, 3 = Moderately easy to chew; Flavour desirability: 6 = Moderatly desirable, 5 = Slightly desirable, and 4 = Slightly undesirable; Foreign flavour : 5 = Slightly intense, 4 = Slightly weak, 3 = Moderately weak.

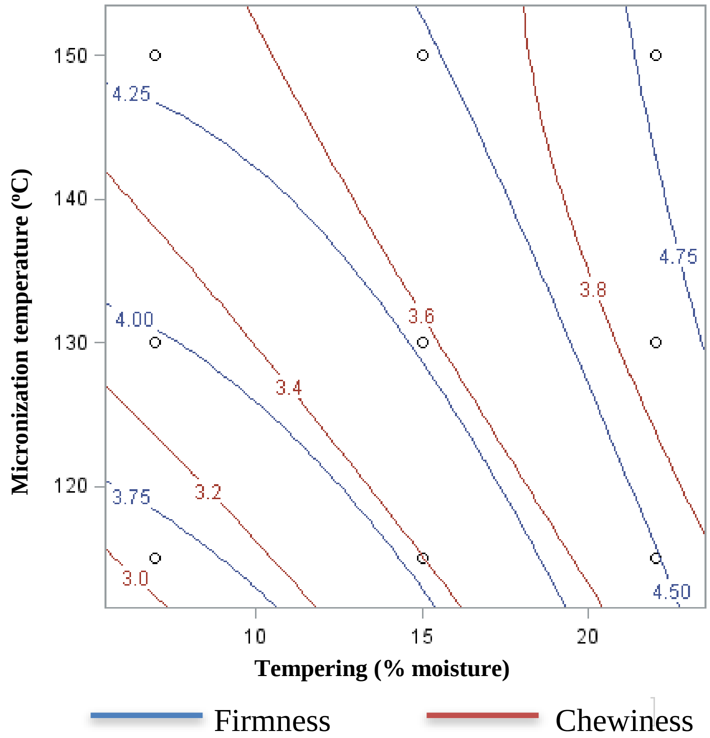

Figure 4.2 Contour plot for sensory firmness and chewiness (n=9). Firmness: 5 = Slightly firm, 4 = Slightly soft and 3 = Moderately soft; Chewiness: 4 = Slightly easy to chew, 3 = Moderately easy to chew.

The sensory firmness increased in a similar pattern as the increase of chewiness scores (Figure 4.1a, Figure 4.1b, and Figure 4.2), as suggested by the high correlation obtained from Pearson correlation analysis. Firmness was considered in this study as it is one of the important properties for bologna. Increased micronized temperatures and initial seed moisture (tempering) resulted in bologna with a firm texture. From the contour plot, the highest firmness resulted when seeds were tempered to more than 20% and then heated to more than 130 °C (Figure 4.1a). Equivalent firmness (for example for scores of 4.5) was possible with tempered to 22% seed moisture and 115°C micronization and tempered to 15% seed moisture and 150°C micronization, and would not be possible without tempering (i.e. at seed moisture levels of 7%).

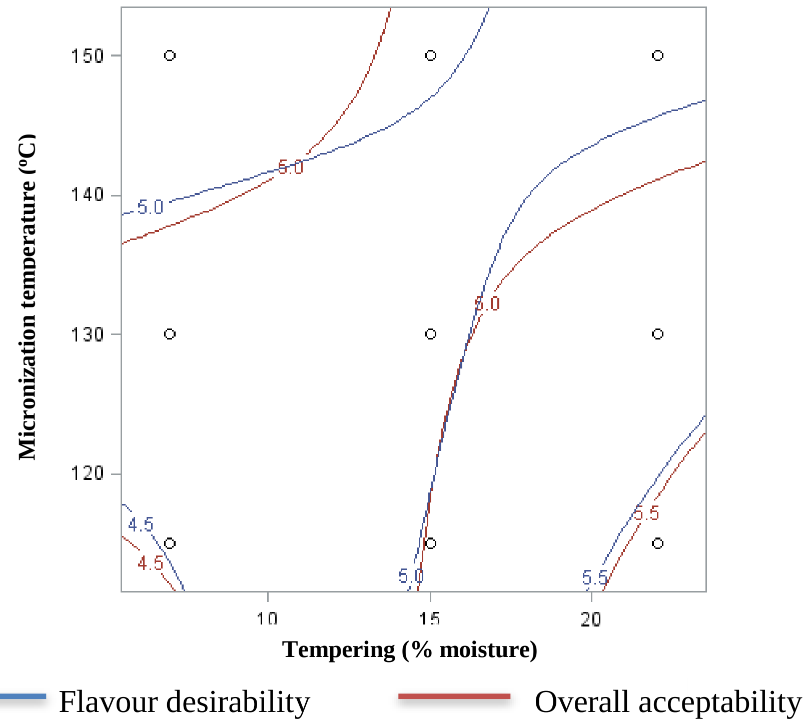

Figure 4.3 Contour plot for flavour desirability and overall acceptability (n=9). Flavour desirability: 6 = Moderatly desirable, 5 = Slightly desirable, and 4 = Slightly undesirable; Overall acceptability: 6 = Moderately acceptable, 5 = Slightly acceptable.

A strong correlation between overall acceptability and flavour desirability also found in the regression model when these properties were combined (Figure 4.3). In addition Figure 4.1 and Figure 4.3 showed that at high seed moisture, a lower micronization temperature (<120^{o}C) was required to produce the bologna which had high flavour desirability and overall acceptability scores, whereas at the low seed moisture, high micronization temperature (>135^{o}C) was needed.

4.5 Summary

Chickpea flours were incorporated as a binder into low-fat pork bologna at level of 5% (w/w). The colour, cooking properties, texture profile properties, sensory properties by trained panel and proximate analysis, including moisture, protein, fat and ash of bologna were investigated. The bologna formulated with chickpea-based, wheat-based, and no binder contained 10.12-10.89% fat and 11.12-11.59% protein. There was no signifiant (p<0.05) difference in the fat content among all bologna treatments, whereas the protein content of wheat-based bologna was significantly (p<0.05) lower than other bologna samples. The moisture content of the no binder treatment was higher than other treatments as the flour was substituted by extra meat (which contained significant moisture) in order to meet the requirement of protein content (11% protein). Although the ability of flour to bind water and oil was shown in the study 1, adding chickpea flour to low-fat pork bologna resulted in no improvement on cook loss, expressible moisture and purge loss. Bologna containing chickpea flour was yellower in colour, based on CIE system and sensory evaluation from trained panel compared to those with added wheat flour or no binder which were similar. The results from texture profile analysis (TPA) showed that adding chickpea flour from seed tempered to 22% seed moisture with 115^{o}C micronized temperature provided the harder texture than those of bologna with added chickpea flours from untempered seed or seed tempered to 15% seed moisture and micronized to 115^{o}C. Similar results was found in sensory evaluation. The effect of seed tempering moisture and micronization of chickpea seed on juiciness was not found in this study which corresponded to the result from WHC of bologna (cook loss, expressible moisture, and purge losses). The difference of flavour intensity among all bologna contained chickpea treatments was not found in sensory evaluation. Bologna containing chickpea flour from seed tempered to 22% seed moisture following 115^{o}C micronization and from untempered seed which was micronized to 150^{o}C showed the significant effect on flavour desirability, foreign flavour and overall acceptability scores, highlighting the importance of appropriate micronization conditions.

4.6 Connection to the next study

It was clear from this study that initial seed moisture levels (tempering conditions) and micronization temperatures of chickpea flours had an effect on the characteristics of the final bologna product when chickpea flours were used as a binder in the model low-fat pork bologna. In order to confirm the optimal micronization temperatures and seed tempering moisture conditions, consumer acceptability of the low-fat pork bologna product containing chickpea flours were investigated in the next study. Moreover, since there was a limitation on overall acceptability on trained panel study, using consumers is a better way to investigate product acceptability. The treatments were chosen based on sensory firmness, foreign flavour, flavour desirability and overall acceptability score to produce a range of properties.

5. Study 3: Consumer panel study of low-fat pork bologna with added chickpea flours

5.1 Abstract

In study 3, a consumer panel was asked to complete a questionnaire providing demographic, consumer perceptual, and behavioural information. Consumer acceptability of the low-fat pork bologna product containing difference binders (5% additional level) was assessed by an 101 member untrained panel. There were 5 bologna treatments involved in this study which were selected based on the results from study 2: a) wheat flour; b) flour from non-micronized chickpea seed; c,d) flour from untempered chickpea seed with micronization to 130 and 150^{o}C; and e) flour from seed tempered to 22% seed moisture with micronization to 115^{o}C. Results of consumer panel evaluations showed that bologna containing chickpea flour had significantly (p<0.05) lower colour acceptability score than bologna prepared with wheat flour, while no significant (p<0.05) differences of bologna colour were found among all bologna with the addition of chickpea flour. Bologna produced with wheat flour showed significantly (p<0.05) higher appearance score than bologna produced with chickpea flour from seed tempered to 22% following 115°C micronization and all bologna containing chickpea flour from untempered seed (micronized and non-micronized), respectively. Chickpea flour from non-micronized seed had the lowest overall texture acceptability score, compared to all bologna treatments. Whereas, flour from untempered chickpea seed micronized to reach 150^{o}C, and chickpea seed tempered to 22% seed moisture micronized to 115^{o}C did not affect overall texture acceptability of bologna, compared to those with wheat flour. Bologna with chickpea flour from seed tempered to 22% following 115^{o}C micronization had the highest overall juiciness acceptability score which was not significant (p<0.05) difference from bologna produced with chickpea flour from seed micronized to 150^{o}C without tempering, whereas these bologna had significantly (p<0.05) higher in overall juiciness acceptability than bologna with wheat flour. Bologna with chickpea flour from non-micronized seed showed the lowest flavour acceptability score. The greater acceptability of flavour score was found when bologna produced with chickpea flour from seed

micronized at 150^{o}C without tempering and seed tempered to 22% seed moisture following 115°C micronization which was not significantly (p<0.05) different from bologna with wheat flour. Overall, consumers rated bologna produced with chickpea flour from 22% seed moisture following 115^{o}C micronization and bologna produced with chickpea flour from seed micronized to 150^{o}C to have comparable overall texture, overall juiciness, flavour, overall acceptability and also willingness to purchase scores to bologna added wheat flour which was used as an industry standard reference.

5.2 Introduction

Sensory properties are an important elements of quality characteristics of food products, determining consumer reaction and satisfaction. In this study, a consumer panel was used to better understand consumer-purchasing behavior as it relates to purchasing comminuted meat products that include pulse-based binders. A consumer panel of 101 participants was asked to complete a questionnaire providing demographic and consumer perception and behavioural information, then evaluate five bologna treatments based on their attributes such as colour, appearance, overall texture, overall, juiciness, flavour, and overall acceptability. The willing to purchase of panelists for each sample was also assessed.

5.3 Materials and methods

5.3.1 Sensory evaluation

Selected treatments of low-fat (<10%) pork bologna from the previous study were assessed for their acceptability by a consumer panel. The chickpea binders chosen for this consumer study were based on sensory results from the trained panel study. This study was accepted on ethical grounds (BEH # 12-159) by the University of Saskatchewan Behavioral Research Ethics Board. There were 5 bologna treatments evaluated in this consumer trial which with different binders added at the 5% level (w/w): 1) wheat flour, 2) flour from non-micronized chickpea seed, 3) flour from untempered chickpea seed with micronization to 130^{o}C, 4) flour from untempered chickpea seed with micronization to 150^{o}C, and 5) flour from seed tempered to a 22% seed moisture with micronization to 115^{o}C. These treatments were chosen based on sensory firmness, foreign flavour, flavour desirability and overall acceptability scores from the trained panel study (study 2). A total of 101 consumers (students or employees of the University of Saskatchewan) were recruited to participate in this study. Consumers were asked to come in one time for 20 min to complete 2 parts of the study. In part 1, panelists were asked to complete a 4- page consumer questionnaire which consisted of questions related to demographics, food purchasing and consumption behavior (Appendix B). In part 2, panelists were asked to taste the bologna samples and rate them using 8-point hedonic scales based on their acceptability of colour, appearance, overall texture, overall juiciness, flavour and overall acceptability (8 = like extremely, 1 = dislike extremely) (Figure 5.1).

Moreover, the willing to purchase of panelists for each sample was asked (1= yes, 2 = no). Sample preparation and serving protocol were the same as used for study 2; except during bologna preparation, the emulsion mill (Type 1E-75F, Alexanderwerk, Remscheid, Germany), which was used in study 2, was replaced by Karl Schnell Maschinenfabrik emulsion mill (Type 012, Winterbach, Germany) in this consumer study. The study was conducted in a sensory room with individual booths and under white lighting. The panelists were asked to clean their palate between samples with water at room temperature and unsalted crackers.

5.3.2 Statistical analysis

The mean and standard deviations for sensory data of all treatments were calculated. Observed data were arranged into a Randomized Complete Block Design using Statistical Package for the Social Sciences (SPSS) software (SPSS 16.0, SPSS, Inc., Chicago, IL). Panelists were considered as a block. The difference between treatments was compared by the least significant difference (LSD) procedure. The significance was declared at $p<0.05$.

Consumer Sensory Evaluation of Pork Bologna

Participant #: ___________.

Instruction: Please first evaluate each sample visually for colour and appearance then taste the samples in the order that the sample codes are presented to you. **Please take a bit of cracker and a drink of water before and between samples**. You can avoid swallowing samples after tasting, is preferred. A waste cup is provided.

Sample code: ____________

How much do you like/dislike the **colour** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **appearance** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **overall texture** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **overall juiciness** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **flavour** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

Considering all the characteristics of these samples, indicate your **overall acceptability**

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

Would you buy this product? (check one)

☐ Yes ☐ No

Comments:……………………………………………………………………………………

……………………**Your comment is highly appreciated. Thank you for your cooperation.**

Figure 5.1 Scorecard for consumer sensory evaluation of pork bologna

5.4 Results and discussion

5.4.1 Consumer demographics and purchasing behavior

The demographics and consumer beliefs and behaviour information of consumers were obtained from Part 1 of the Questionnaire (Appendix B). This information was collected in order to gain a basis for further consumer segmentation and to understand consumer familiarity with meat products prior to their participation. Table 5.1 presents consumer data relating to household and demographic information. Results show that there were 55% females and 45% males involved in this study with the majority between 20 to 29 years old (42%). The sex distribution of panelists closely reflected that of the Canadian population. Statistics Canada (2012) reported that in 2012, 17.2 million females accounted for 50.4% of the total population, moreover the female majority would continue for the next 50 years. However, the age distribution of panelists in this study did not represent the Canadian population. Among Canadian population, 14.03% were in the age group of 20 to 29. The number of people aged 30 to 39, 40 to 49, 50 to 59, and 60 to 69 accounted for 13.52%, 14.52%, 14.71% and 10.63% of the Canadian population, respectively (Statistics Canada, 2012). About half (51%) of the consumer panel had completed graduate school, while 31.68% held university degrees. The majority (49%) of the consumer panel was of Asian descent following by 22% North America, 15% European, and 15% originating from other regions such as Africa, Central or South America. Statistics Canada (2009) reported that ethnic origins of people in Canada was 32.22% Canadian, 21.03% British Isles origins, 15.82% French, 15.11% Scottish, 13.94% Irish, 10.18% German and others were from a minority group, 43.38%. Almost half of visible minorities population in Canada are comprised of immigrants of Chinese descent and South Asian (Statistics Canada, 2012). Therefore, the results from this study may not be able to represent the Canadian population since ethnic backgrounds affect the food consumption and purchase behavior.

Table 5.1 Consumer demographic data (n=101).

Question	Response	Percent (%)
Gender	Male	45
	Female	55
	Total	100
Age	20 - 29 Years	42
	30 - 39 Years	28
	40 - 49 Years	19
	50 - 59 Years	10
	60 - 69 Years	1
	Total	100
Education	High School Graduate	4
	Some University	13
	University/ College graduate	32
	Graduate School	51
	Total	100
Ethnicity	European	15
	North American	22
	First nation	1
	Asian	49
	African	8
	Central/South American	3
	Other	2
	Total	100
Household Size	1	24
	2	28
	3	17
	4	20
	5	9
	6	2
	Total	100
Children Number	0	73
	1	13
	2	10
	3	4
	Total	100
What is your role in grocery shopping for your household?	Primary shopper	64
	Share the shopping	26
	Someone else is the primary shopper	10
	Total	100

Table 5.1 Continued…

Question	Response	Percent (%)
Household Income	Under $20,000	29
	$20,000 - 39,000	11
	$40,000 - 59,000	20
	$60,000 - 79,000	16
	$80,000 - 99,000	16
	Over $100,000	8
	Total	100
How aften do you consume bologna?	3 - 5 times per week	2
	1 - 2 times per week	12
	1 - 2 times per month	43
	I don't eat bolognas typically	43
	Total	100
What fat level would you typically buy?	Regular fat	44
	Low fat	36
	I don't buy bologna	20
	Total	100
What salt level would you typically buy?	Regular salt	44
	Low salt	36
	I don't buy bologna	20
	Total	100
What type of bologna do you prefer to buy?	Pork	48
	Beef	11
	Chicken	12
	Made from several meats	17
	other	12
	Total	100

The number of persons living in a household ranged from 1 to 6 and the greater number of the households (73%) did not have any children less than 18 years old. More than half (64%) of the consumer identified themselves as the primary shoppers in theirs household and 25.74% stated that they shared the shopping. About 29% of the panel had annual household incomes lower than $20,000, while 31% and 32% of consumers had gross annual incomes between $20,000-59,000 and $60,000-99,000, respectively. There was 43% of consumers that consumed bologna 1-2 time per month, and 43% stated that they do not eat bologna typically. The majority of respondents (44%) stated that they preferred to purchase regular fat and regular salt bologna, while 35.64% of consumers looked for low fat and low salt bologna and 19.80% indicated that bologna are not typically consumed. About half (48%) of the respondents stated that they preferred to buy pork bologna than bologna made from other meat sources such as beef and chicken.

5.4.2 Consumer values

(a) Product features

Table 5.2 shows various important factors and consumer ratings. The 6-point scale was reduced into 3 categories whereas 1 and 3 indicated low important and high important, respectively.

Table 5.2 Consumer statements on importance of product features when shopping for bologna (n = 101).

Purchasing factors	**Importance[1]**			
	Low (%)	**Medium (%)**	**High (%)**	**Total (%)**
Price	5	62	33	100
Fat content	8	62	30	100
Salt content	11	48	41	100
Additive exclusion	14	46	40	100
Flavour variety	12	40	48	100
Made from specific meat	23	42	35	100
Gluten-free	73	21	6	100

[1]Reduced to 3-point scale of importance from the original 6-point scale.

It is essential to understand the significant factors that consumers use to decide when purchasing products since it is helpful for product development. Those factors having greatest importance should be considered as they might have a significant effect on consumer's choice. In this study, the majority of respondents indicated the variety of flavour to be the factor having the greatest importance, with price, fat content, salt content, exclusion of additives, and bologna made from specific meat having medium importance (Table 5.2). However, majority of respondents (~73%) indicated gluten-free option having the least importance, about 27% of the panelists considered gluten-free bologna to have medium or high importance. This information gives an idea for the industry to consider gluten-free meat products in the stages of new product development. According to Health Canada (2012), celiac disease is now recognized as one of the most common chronic diseases in the world with an increasing number of people being diagnosed. It is estimated that 1 out of every 100 people in Canada are affected by celiac disease (Canadian Celiac Association, 2013). The National Foundation for Celiac Awareness (2013) reported that about 15-25% of consumers around the world are looking for gluten-free products. The gluten-free food industry is expected to grow continuously, reaching $6 billion by 2015. Moreover, The National Restaurant Association (NRA) and The American Culinary Federation (ACF) have named “gluten-free” as one of the top food trends for 2011 (National Foundation for Celiac Awareness, 2013).

(b) Beliefs and lifestyle

Table 5.3 presents the indicators of consumer belief and behavior in terms of health, nutrition, and food purchase based on a 3-point scale. This scale (1 = disagree, and 3 = agree) has been reduced from an original 6-point scale for ease of review. The information obtained from this study showed that not only reducing fat level and demand for nutritious food options, but also flavor quality, trend of having a healthier lifestyle, and a positive perception of chickpea and pork could lead to potential marketability of a low-fat pork bologna containing chickpea.

There was 46% and 47% of consumers that considered themselves health conscious and who exercised on a regular basis, respectively. The majority of respondents indicated that they regularly read food nutrition labels (63%), were willing to pay more for more nutritious foods (55%), and would choose lower fat options when available (53%). Agriculture and Agri-Food Canada (2010) reported that 72% of U.S. consumers were more concerned with food quality than price. Moreover, Canadian consumers are paying attention to the reduction of fat, salt, cholesterol and calories. Even though about half of consumers would like to buy low fat product, flavour quality for reduced fat products is important since 23% of panel said they disagree with buying low fat product with lower flavour quality, while 57% was neutral and 20% agreed to choose the products. About half (53%) of the consumer panel stated that price is moderately important factor when buying meat products. For the consumer’s beliefs, about half (49%) thought that chickpea is a good source of nutrition, and 59% believed that pork is of neutral importance as a source of nutrition. Moreover in the consumer study of lentil and its utilization in beef burgers, Der (2012) found 74 and 65% of consumers believed that lentil and beef, respectively, are good sources of nutrition.

Table 5.3 Consumer belief and behavioral data (n=101).

Statement	Agreement level			Total (%)
	Disagree (%)	Neutral (%)	Agree (%)	
I consider myself health conscious.	0	54	46	100
I exercise regularly.	6	47	47	100
I read food nutritional labels regularly.	9	28	63	100
I will pay more for nutritious foods.	9	36	55	100
Price is most important factor when buying meat products.	24	53	23	100
I choose lower fat versions of food when available.	14	34	52	100
I choose lower fat versions of food when available even at the expense of lower flavour quality.	23	57	20	100
I believe pork is a good source of nutrition.	7	59	34	100
I believe that chickpea is a good source of nutrition.	5	46	49	100

5.4.3 Sensory score for low-fat pork bologna

To determine the level of sensory related to acceptability, the five bologna treatments were evaluated with a 101 untrained consumer panel. Scales used in this study were 8 to 1, which 8 indicated “like extremely”, while 1 referred to “dislike extremely”. Mean values for colour, appearance, overall texture, overall juiciness, and flavour acceptability are presented in Table 5.4. The effects of seed tempering moisture condition and micronization temperature on the colour acceptability of bologna with chickpea flour were not found in this study since the colour of all bologna with chickpea flour was creamy-yellow (personal observation). Whereas, pork bologna containing wheat flour as a binder had a significantly (p<0.05) higher acceptability value in colour (5.51) than bologna prepared with chickpea flour (4.58-4.86). This result corresponded to the result from the trained panel study since no significant (p<0.05) differences in bologna colour were found among all bologna produced with chickpea flour. Moreover, trained panelists found that bologna produced with wheat flour had significantly (p<0.05) more pinkish red colour than bologna containing chickpea flour, which may be a reason that bologna prepared with wheat flour had significantly (p<0.05) higher colour acceptability scores, compared to those with chickpea flour. In addition, the colour results obtained from the CIE Lab system showed that bologna contained chickpea flour was significantly (p<0.05) higher in yellow colour than bologna produced with wheat flour.

The overall appearance of a food product is important since consumers often assesses the quality of product by its appearance, as its primary indicator of food quality. The appearance properties of a product consist of various visual properties, including colour, size, shape, and surface texture (Kildegaard et al., 2011). Bologna produced with wheat flour showed significantly (p<0.05) higher acceptability in appearance score (5.55) than bologna produced with chickpea flour from seed tempered to 22% following 115°C micronization (5.03) and bologna containing chickpea flour from untempered seed (4.51-4.73), respectively. There was no significant (p<0.05) difference of appearance acceptability scores due to chickpea processing for those bologna made with flour from non-micronized or untempered chickpea seed with 130 and 150^{o}C of micronization. In this study, the colour of bologna containing chickpea flour from seed tempered to 22% following 115°C micronization did not lead to the higher appearance acceptability score since no differences were found in colour among all chickpea treatments.

For the overall texture acceptability, bologna with chickpea flour from seed tempered to 22% seed moisture and micronized to 115^{o}C had the highest texture acceptability score (5.38) which was also significantly (p<0.05) higher than bologna with chickpea flour from non-micronized seed (4.38) and seed micronized to 130^{o}C without tempering (4.96). Whereas, there was no significant (p<0.05) difference in overall texture acceptability among bologna containing wheat flour (5.31), chickpea flour from untempered seed with micronized to reach 150^{o}C (5.20) and chickpea flour from seed tempered to 22% seed moisture and micronized to 115^{o}C (5.38). In general, results for overall texture acceptability were consistent to the sensory evaluation by the trained panel and instrumental texture measurement since there were no clear differences on overall texture acceptability, firmness score (trained panel), and TPA-hardness among bologna containing chickpea flour from micronized seed, while bologna produced with chickpea flour from non-micronized seed had the lowest score on these measurements. Instrumental texture measurement and consumer test showed that bologna produced with chickpea flour from seed micronized to 150^{o}C without tempering and bologna containing chickpea flour from seed tempered to 22% seed moisture following 115^{o}C micronization had comparable liking of hardness to the wheat flour treatment, this result was not found in sensory evaluation of trained panel. This inconsistency may be due to the trained panelists being more sensitive than consumers.

Bologna containing chickpea flour from seed micronized at 115^{o}C after tempered to 22% initial seed moisture had the highest overall juiciness acceptability score (5.62) which was not significantly (p<0.05) different from bologna produced with chickpea flour from seed micronized to 150^{o}C without tempering (5.39). However, bologna prepared with chickpea flour from seed tempered to 22% seed moisture following 115^{o}C micronization was significantly (p<0.05) higher in juiciness acceptability than bologna with wheat flour (5.14), chickpea flour from non-micronized seed (5.25) and that with chickpea flour from micronized to 130^{o}C without tempering (5.21). The overall juiciness acceptability obtained from the consumer study was difference from the sensory results from study 2. The trained panel did not find the difference of juiciness intensity among all bologna with the presence of binders, whereas addition of chickpea flour from seed tempered to 22% seed moisture prior to micronized to reach 115^{o}C and chickpea flour from seed micronized to 150^{o}C without tempering into low-fat pork bologna improved juciness liking, compared to bologna containing wheat flour.

Table 5.4 Mean acceptability scores for five bologna treatments. Data represent the mean ± one standard deviation (n = 101).

Treatment	**Acceptability[1]**				
	Colour	**Appearance**	**Overall Texture**	**Overall Juiciness**	**Flavour**
Wheat Flour	5.51 ± 0.90^{a}	5.55 ± 0.83^{a}	5.31 ± 1.02^{a}	5.14 ± 1.12^{b}	5.23 ± 1.15^{a}
Chickpea flour (Untempered)					
Non-micronized	4.58 ± 1.14^{b}	4.51 ± 1.05^{c}	4.83 ± 1.12^{c}	5.25 ± 1.11^{b}	4.42 ± 1.30^{b}
Micronized to 130°C	4.66 ± 1.13^{b}	4.68 ± 1.03^{c}	4.96 ± 1.11^{bc}	5.21 ± 1.08^{b}	4.67 ± 1.44^{b}
Micronized to 150°C	4.67 ± 1.04^{b}	4.73 ± 1.04$_{c}$	5.20 ± 1.08^{ab}	5.39 ± 1.08^{ab}	5.29 ± 1.18^{a}
Tempered to 22% moisture					
Micronized to 115°C	4.86 ± 1.13^{b}	5.03 ± 1.05^{b}	5.38 ± 0.92^{a}	5.62 ± 0.89^{a}	5.45 ± 0.97^{a}

$^{a\text{-}c}$ Means within the same column with the same letter are not significantly different (p>0.05)
[1] Highest possible score = 8 (Like Extremely); Lowest possible score = 1 (Dislike Extremely)

The acceptability of flavour was significantly (p<0.05) affected by temperature when the chickpea seed was not tempered. Bologna with chickpea flour from non-micronized seed showed the lowest flavour acceptability score (4.42) and it was not significantly (p<0.05) different from the score of chickpea flour from untempered seed with micronization at 130^{o}C (4.67). The greater acceptability of flavour score was found when higher micronization temperature was applied. Bologna prepared with chickpea flour from untempered seed that was micronized to 150^{o}C had a significantly (p<0.05) higher flavour acceptability score than from comparable products made with chickpea flour from seed micronized to 130^{o}C at the same tempering condition. In addition, there was no significant (p<0.05) effect on panelist flavour acceptability scores among the bologna produced with wheat flour (5.23), chickpea flour from untempered seed following micronization to 150^{o}C (5.29) and chickpea flour from seed tempered to 22% seed moisture following 115°C micronization (5.45).

The effects of having plant-based binders in meat products on sensory characteristics were observed in many studies. Prinyawiwatkul et al. (1997) found that flavour acceptability of nuggets containing cowpea and peanuts flour decreased with increased amount of flour in the formulations. Der (2012) found flavour acceptability was higher in burgers prepared with 6% lentil flour from seed micronized to 130^{o}C compared with those with lentil flour from non-micronized seed. Whereas, in this study no significant (p<0.05) affect of micronization on flavour acceptablity was found between bologna containing 5% chickpea flour from non-micronized seed and 130^{o}C micronized seed without tempering, but micronized seed to 150°C did result in significantly (p<0.05) higher flavour acceptability. In agreement with the results of this study, Shariati-Ievari (2013) reported that low-fat beef burgers containing 6% chickpea flour from seed micronized at 150^{o}C had higher flavour acceptability scores compared to those containing chickpea flour from micronized seed at 130^{o}C. Micronization temperatures had an inverse relationship with the concentration of beany aroma compounds of chickpea and may be considered as an effective method to reduce the activity of LOX isozymes (Shariati-Ievari, 2013).

The overall acceptability and willingness to purchase scores are shown in Table 5.5. Bologna containing chickpea flour from seed tempered to 22% seed moisture prior to micronization to reach 115^{o}C received the highest overall acceptability score (5.38) although there was no significant (p<0.05) difference among bologna containing wheat flour (5.28) and bologna prepared with chickpea flour from untempered seed with micronized to 150°C (5.20). Adding chickpea flour from non-micronized seed into the bologna leaded to the lowest overall acceptability score (4.61). The mean score for acceptability of colour was the same for bologna with chickpea flours from micronized and non-micronized seed, however consumers rated overall texture, overall juiciness and flavour of bologna containing chickpea flour from micronized at 150°C and chickpea flour from seed tempered to 22% seed moisture following 115°C more acceptable than bologna with flour non-micronized seed. These results indicated that texture, juiciness, and flavor have a considerable effect on overall acceptability of bologna containing chickpea flour. Shariati-Ievari (2013) reported that aroma and flavor of burgers containing chickpea flour from seed micronized at 150°C at the level of 6% were more acceptable compared to those containing chickpea flour from non-micronized seed, these sensory properties may have an effect on overall acceptability.

Table 5.5 Overall acceptability and willingness to purchase scores for five bologna treatments. Data represent the mean ± one standard deviation (n = 101).

Treatment	**Overall Acceptability**[1]	**Willingness to Purchase**[2]
Wheat Flour	5.28 ± 0.98^{ab}	1.39 ± 0.49^{c}
Chickpea flour (Untempered)		
Non-micronized	4.61 ± 1.00^{c}	1.77 ± 0.42^{a}
Micronized to 130°C	4.96 ± 1.14^{b}	1.65 ± 0.48^{ab}
Micronized to 150°C	5.20 ± 0.98^{ab}	1.50 ± 0.50^{bc}
Tempered to 22% moisture		
Micronized to 115°C	5.34 ± 0.92^{a}	1.36 ± 0.48^{c}

a-c Means within the same column with the same letter are not significantly different (p>0.05)
[1] Highest possible score = 8 (Like Extremely); Lowest possible score = 1 (Dislike Extremely)
[2] 1 = yes; 2 = no

The consumers rated bologna produced with chickpea flour from seed tempered to 22% seed moisture following 115°C micronization with the lowest willingness to purchase score (1.36), which actually indicates the highest willingness to purchase. Whereas, bologna containing chickpea flour from non-micronized seed showed the highest willing to purchase score, which actually indicates the lowest willingness to purchase. No significant (p<0.05) differences of willingness to purchase score was found among bologna containing wheat flour, chickpea flour from untempered seed with micronized to 150°C and chickpea flour from seed micronized to 115°C with 22% initial seed moisture.

The frequency of overall acceptability and willingness responses for each category by treatment are shown in Table 5.6 and Table 5.7. About 8% of the consumers stated that they moderately and very much dislike bologna containing chickpea flour from non-micronized seed, 12% of consumer moderately and very much dislike bologna produced with chickpea flour from seed micronized to 130°C without tempering (Table 5.6). Both treatments received comments of being mushy, having soft texture and undesirable flavour, whereas these comments were not found in other bologna samples.

The lower frequency of a "yes" response to the question of willingness to purchase bologna containing chickpea flour from non-micronized seed might due to the fact that this treatment had a soft texture and strong foreign flavour (from study 2), moreover, it also had the lowest acceptability score for texture, flavour and overall acceptability in this consumer study. The soft texture of bologna containing chickpea flour from non-micronized seed may be due to the chickpea flour had low water holding and oil absorption capacity (Table 3.5). Fasina et al. (2001) and Mwangwela et al. (2007) stated that micronized seed was less dense than non-micronized seed which might play an essential role in higher water absorption and higher oil holding capacity of flour. Moreover, about 83% of consumers stated that they "liked" (with extremely, very much, moderately and slightly like combined) bologna containing chickpea flour from seed tempered to 22% seed moisture following 115°C micronization and 76% of consumers "liked" bologna produced with chickpea flour from seed micronized at 150°C. These results showed that bologna containing chickpea flour from seed tempered to 22% seed moisture following 115°C micronization was comparable in overall acceptability with bologna added with wheat flour since about 84% of consumers showed that they "liked" bologna produced with wheat flour. Whereas, about 49% and 59% of consumers stated that they "liked" bologna containing chickpea flour from non-micronized seed and chickpea flour from seed micronized at 130°C, respectively.

Table 5.6 The frequency of overall acceptability responses for each category by treatment (n = 101).

Treatment	**Scales**								
	Like extremely	Like very much	Like moderately	Like slightly	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely	Total
Wheat Flour	0	9	36	39	12	3	1	0	101
Chickpea flour (Untempered)									
Nonmicronized	0	2	20	27	44	5	3	0	101
Micronized to 130^{o}C	0	16	20	23	30	10	2	0	101
Micronized to 150^{o}C	3	6	28	39	23	2	0	0	101
Tempered to 22% moisture									
Micronized to 115^{o}C	3	9	32	39	14	1	3	0	101

Table 5.7 The frequency of willing to purchase responses by treatment (n = 101).

Treatment	**Willing to purchase**		
	Yes	**No**	**Total**
Wheat Flour	62	39	101
Chickpea flour (Untempered)			
Non-micronized	23	78	101
Micronized to 130°C	35	66	101
Micronized to 150°C	49	52	101
Tempered to 22% moisture			
Micronized to 115°C	65	36	101

(a) Consumer segmentation

Consumer segmentation by gender showed that hedonic acceptability scores varied by gender for the five bologna treatments. The mean acceptability scores categorized by gender for bologna treatments are presented in Table 5.8.

Table 5.8 Mean acceptability scores for low-fat pork bologna containing various binders according to gender (n = 101).

Gender	**Percent of panelists**	**Bologna Treatment[1,2]**				
		WF	**NM**	**M130**	**M150**	**T115**
Male	45.54	5.22 ± 0.99^{a}	4.63 ± 0.97^{b}	5.02 ± 1.09^{ab}	5.28 ± 0.93^{a}	5.30 ± 0.94^{a}
Female	54.46	5.33 ± 0.98^{a}	4.60 ± 1.03^{c}	4.89 ± 1.18^{bc}	5.13 ± 1.02^{ab}	5.36 ± 0.91^{a}

[a-c] Means within the same row with the same letter are not significantly different (*p*>0.05)
[1] Highest possible score = 8 (Like Extremely); Lowest possible score = 1 (Dislike Extremely)
[2] WF = wheat flour, NM = chickpea flour from non-micronized seed, M130 = chickpea flour from untempered chickpea seed which was micronized to reach 130°C, M150 = = chickpea flour from untempered chickpea seed which was micronized to reach 150°C and T115 = chickpea flour from chickpea seed tempered to 22% following 115°C micronization

Both male and female consumers gave bologna prepared with chickpea flour from non-micronized seed the lowest acceptability scores among all bologna treatments. Males and females found no significant (p<0.05) differences in overall acceptability between wheat flour bologna, bologna containing chickpea flour from untempered seed with micronized at 150^{o}C, and bologna produced with flour from chickpea seed micronized at 115^{o}C after tempered to 22% initial seed moisture, while these treatments had significantly (p<0.05) more acceptable than bologna containing chickpea flour from non-micronized seed. Females found bologna with wheat flour were more acceptable (5.33) than bologna produced with chickpea flour from untempered seed which was micronized to 130^{o}C (4.89), whereas males generally found no difference between these two bologna treatments. The study showed that gender had an effect on food preferences. This would be an advantage since understanding factors that influences consumer's responses to the food is needed for marketing strategists.

5.5 Conclusion

A consumer panel of 101 participants was asked to evaluate five bologna treatments containing wheat flour, chickpea flour from non-micronized seed, chickpea flours from untempered seed which were micronized to 130^{o}C and 150^{o}C, and chickpea flour from seed tempered to 22% following 115°C micronization. Moreover, demographic, perceptual, and behavioural information of these consumers were assessed. This study confirmed the excellent capability of tempering along with micronization to improve the sensory characteristics of low-fat pork bologna. Overall, the addition of 5% chickpea flour from untempered seed with micronized to 150°C and chickpea flour from seed tempered to 22% following 115°C micronization in the bologna's formulation increased acceptability of overall texture, flavour and overall acceptability compared to bologna with the addition of chickpea flour from non-micronized and untempered chickpea seed which were micronized to 130^{o}C. Furthermore, these treatments were comparable with bologna prepared with wheat flour. Therefore, this study suggests that micronization improves the sensory characteristics of low-fat pork bologna. At high seed moisture content, low micronization temperature was needed to produce the bologna which had high acceptability of flavour, overall texture, overall juiciness, and overall acceptability scores, whereas at the low seed moisture, high micronization temperature was required. Consumer found the overall juiciness of bologna containing chickpea flour from seed tempered to 22% seed moisture prior micronized to115^{o}C was juicier than wheat flour bologna, but it was not different from bologna prepared with chickpea flour from untempered seed micronized to

150°C. Addition of chickpea flour into bologna caused less colour acceptability compared to those containing wheat flour. The mean acceptability scores of bologna treatments categorized by gender showed that when gender is taken into account, males found bologna containing wheat flour and chickpea flour from untempered seed micronized to 130°C to have similar overall acceptability, whereas females found bologna produced with chickpea flour from untempered seed micronized to 130°C were less acceptable than wheat flour bologna. Moreover, the information obtained from this study showed that consumers were interested in buying the low-fat product and they were willing to pay more for nutritious foods while maintaining flavour quality should be considered. Consumers believed that chickpea and pork are a good source of nutrition therefore developing meat (pork) product containing chickpea could have a potential marketability.

6. GENERAL DISCUSSION

The overall goal of this research was to examine the possible use of chickpea flour, along with the effect of seed tempering moisture and micronizing temperatures, as a binder in a model low-fat pork bologna product. Chickpea is an economically important crop to Saskatchewan and Canada, and represents a nutritious source of protein, fibre, carbohydrates and minerals. The use of plant-based flours in comminuted meat products is attractive to the meat industry in order to reduce prices and to enhance functionality.

In the first study (Section 3.0), the effect of tempering (i.e., untempered (7% seed moisture), tempered to a 15% seed moisture level, and tempered to a 22% seed moisture level) and micronization temperatures (115, 130, 150, and 165^{o}C) on the physciochemical and functional properties of Kabuli-type chickpea flour were investigated. These effects were measued by analyzing chickpea flour from non-micronized and micronized seed (tempered and untempered) for their proximate composition, colour, degree of gelatinization, lipoxygenase activity, water holding and oil absorption capacity, and their pasting properties using a rapid visco analyzer (RVA). Findings indicated that the colour of chickpea flour became darker with higher seed moisture and micronization temperature possibly as the result of the heated seed coat and may be due to the Maillard browning reaction involving reducing sugars and proteins within the flour (Alajaji & EL-Adawy, 2006). However the effect of micronization temperature on yellow colour of chickpea flour from untempered seed and seed tempered to 15% seed moisture was not found in this study. Cenkowski & Sosulski (1996) and Scanlon et al. (2005) reported that in order to avoid Maillard browning of legumes during micronization, micronization temperature and level of seed moisture need to be controlled. Since initial seed moisture was low (untempered (7% moisture) and 15% moisture) and the micronized time was short, chickpea seeds did not receive sufficient heat to allow reactions to occur. Moreover, it was also observed in this study that the presence of excess moisture and heat are necessary for starch granules to become gelatinized. Chickpea flour from seed tempered to 22% seed moisture followed by micronization was found to have gelatinized starch content ranging from 8% to 40%, where the percentage increased (p<0.05) as the micronized temperatures were raised. In contrast, there was no gelatinized starch found in other chickpea flour treatments (i.e., chickpea flour from non-micronized seed, untempered chickpea seed and chickpea flour from seed tempered to 15% seed moisture following micronization to 115, 130, 150, and 165ºC). Excess moisture during micronization also had an effect on increasing the water holding (WHC) and oil absorption capacities (OAC). In this study, chickpea flour from micronized seed with and without tempering significantly (p<0.05) improved WHC with the exception of chickpea flour from untempered chickpea seed which when seed micronized to 115ºC showed a similar result as chickpea flour from non-micronized seed. At corresponding micronization temperatures, chickpea flour from seed tempered to 22% seed moisture had significantly (p<0.05) higher WHC and OAC than flour from seeds that were tempered to 15% initial seed moisture and untempered. According to Scanlon et al. (2005) and Ma et al. (2011), micronization at seed moisture content up to 25% was presumed to lead to the swelling of starch granule, to allow of leaching of the amylose polymers from the granules, moreover hydrophobic residues from the interior of protein molecules were most likely exposed as proteins were presumed to undergo partial or complete denaturation. In addition, it can be hypothesized that a greater seed porosity resulting from heating at sufficient seed moisture allows greater entrapment of water and/or fat resulting in higher WHC and OAC. As seed moisture content increases, seed porosity increases (Fasina et al., 2001; Damodaran, 2008). The faster movement of moisture through pores during micronization leads to increased physicochemical changes in starch and protein induced an improvement in water and oil absorption in the flour (Scanlon et al., 2005).

Furthermore, pasting properties of chickpea flour was investigated using rapid visco analyzer (RVA) as part of study one. The data showed that peak viscosity and final viscosity of all untempered chickpea flour increased with an increase in temperature, whereas, peak viscosity and final viscosity showed the opposite trend with temperature when seeds were tempered. In case of the untempered seeds (low seed moisture), the increase in viscosity may occur since the quantity of water in the seeds is less, which would lower the amount of leaching of amylose out of the granules. Upon reheating by RVA, water was added to flour, the starch granules are swollen and opened up which make the amylose/amylopectin can easily leach out of granule contributing to an increase in viscosity. When tempering (moisture) occurred, the higher amounts of water helped stabilize the protein via hydrogen bonding presumably leading to less denaturation of the proteins and more amylose leaching from the granules during micronization. As the system is re-heated, the interconnected amylose network breaks down causing a decline in viscosity.

Micronization was also found to be effective in reducing lipoxygenase (LOX) enzyme activity in chickpea flour. Lipoxygenase enzymes are generally found in legumes which are thought to be responsible for lipid-derived off-flavours since LOX enymzes can oxidize polyunsaturated fatty acids into aldehydes and alcohols (Sessa, 1979). Lipoxygenase activity was found in chickpea flour from non-micronized seed and flour from untempered chickpea seed which was micronized to 115^{o}C to be 1.98×10^{5} units/g of protein and 1.12×10^{5} units/g of protein, respectively. No activity was found in any other treatments possibly due to the micronization conditions including seed tempering moisture (15% and 22% seed moisture content) and micronized temperature (115^{o}C, 130^{o}C, 150^{o}C and 165^{o}C) since heat of micronization impacted the protein structures of the enzymes, causing inactivity. The decrease in LOX enzyme activity may lead to a decrease in off-flavours and an increase in overall acceptability of pulse-based products (Emami & Tabil, 2002).

The second study (section 4.0) was performed in order to examine the potential for incorporating chickpea flours into a low fat pork bologna product, and to measure the effects on their physical, chemical, and sensory attributes relative to those prepared using wheat based binder and a no binder control. The chickpea flour from seed which was micronized to 165^{o}C (untempered, tempered to 15% and 22% seed moisture) was not included in this study as it showed a strong undesirable off flavour within preliminary sensory studies, possibly caused by burnt seed coat and Maillard reactions. The prepared bologna formulated contained 11% protein and 10% fat, which meets Canadian Meat Inspection Regulations for minimum meat and total protein content in cooked sausages (i.e., minimum of 9.5% meat protein and 11% total protein). This study found that the development of low-fat pork bologna can be enhanced with the use of binder based on its ability to bind more water. The low-fat pork bologna that contained wheat flour as a binder had the lowest cook loss, expressible moisture and purge losses than bologna produced with chickpea flour and no binder treatment, respectively. Although the ability of chickpea flour to bind water and oil after treated by tempering and micronization was shown in the study 1 in which chickpea flour from seed tempered to 22% seed moisture followed by micronization resulted in significantly (p<0.05) higher WHC, compared at corresponding temperatures and also flour from seed tempered to 22% moisture before micronization enhanced OAC of chickpea flour compared with chickpea flour from micronized seed at 15% initial seed moisture and untempered chickpea seed, low-fat pork bologna with added chickpea flour showed no improvement on cook loss, expressible moisture and purge loss relative to all chickpea treatments, showing no benefit of tempering and micronization on these attributes. The high WHC of bologna containing wheat flour may be due to the high-carbohydrate content of flour itself which lead it to be highly water absorbent. In general, bologna containing chickpea flour was yellower in colour, based on CIE system and sensory evaluation from a trained panel compared to those with added wheat flour or no binder which were similar. No significant (p<0.05) differences in bologna colour was found among all treatments containing chickpea, showing no benefit of seed tempering moisture and micronization temperature to colour among the processing conditions. Despite the colour of chickpea flour was found to be darker with higher seed moisture and micronization temperatures (study 1), a similar effect was not seen within the model low-fat pork bologna possibly as the result of other ingredient interactions impacting the colour of final product.

The second study, also showed that the types of binders added to the bologna influenced the texture of the end products. Bologna without binders was found to have significantly (p<0.05) lower hardness than bologna with wheat flour based on instrumental texture analysis, TPA-hardness, torsion-shear stress, and the trained panelists score. Among bologna containing binders, bologna produced with chickpea flour from non-micronized seed and untempered seed which was micronized to 115^{o}C had low hardness which was observed by both instrumental texture and sensory analysis. Interestingly, hardness from TPA-hardness of low-fat pork bologna with chickpea flour from seed micronized at 130 and 150^{o}C without tempering, seed tempered to 15% and 22% moisture following 115, 130, 150^{o}C micronization was comparable to bologna containing wheat flour, whereas this result was not found in during sensory evaluation by the trained panel. This inconsistency may be due to the sensory evaluation of trained panelists were more sensitive than instrumental texture analysis. However, the results from study one showed that chickpea flour from seed tempered to 22% seed had higher WHC and OAC than from seeds that were tempered to 15% initial seed moisture and untempered, at corresponding micronization temperature, which is hypothesized to lead to the harder texture.

In terms of the sensory attributes, bologna without binder had the greatest juiciness among panelists, and was significantly (p<0.05) higher than treatments with wheat flour and all bologna produced with chickpea flour. The high juiciness score was found in the no binder treatment which may be caused by the high amount of moisture in the product (Table 4.3). Among all bologna with the addition of binders, the bologna without binder had the highest expressible moisture and purge losses (Table 4.4). Results also showed that both seed tempering moisture and micronization temperature had no effect on flavour intensity. Bologna containing chickpea flour from seed micronized to 150^{o}C without tempering and chickpea flour from seed tempered to 22% following 115^{o}C micronization was found to have higher flavour desirability and overall acceptability scores than bologna with added wheat flour, showing that these chickpea flours have the potential for use as a binder in low-fat meat products. The absence of off-flavour development in bologna containing chickpea flour could be associated with LOX enzyme activity since there was no activity found in all chickpea flours except chickpea flour from non-micronized seed and seed micronized at 115^{o}C without tempering

In the third study, a consumer study (section 5.0) was performed in order to better understand consumer-purchasing behavior as it relates to purchasing of a model comminuted meat product that include chickpea flours as binders. The treatments were chosen from study two based on sensory firmness, foreign flavour, flavour desirability and overall acceptability score. In general, the acceptability of bologna containing chickpea flour was influenced by texture, juiciness, and flavour. The consumers rated bologna with the addition of 5% chickpea flour from untempered chickpea seed following 150°C micronization and chickpea flour from seed tempered to 22% following 115°C micronization in the bologna's formulation to have more overall texture, overall juiciness and flavour acceptability than bologna with chickpea flour from non-micronized seed, moreover these treatments were comparable to bologna containing wheat flour. The colour of all bologna with added chickpea flour was creamy-yellow which corresponded to the result from trained panel study. The no binder treatment was found to have the highest colour acceptability score that might due to bologna without binder had more pinkish red colour (trained panel sensory).

In summary, from this thesis research it was found that chickpea flour is a good alternative to wheat flour. However, it is important to control tempering conditions and micronization temperatures in treated chickpea flours since both factors can have an effect on the final products. Excess moisture during micronization lead to gelatinized starch of chickpea flour resulting in increased WHC and OAC. Moreover, micronization was found to reduce lipoxygenase activity in chickpea flour which had a great effect on sensory properties. At high seed moisture content, low micronization temperature was required, whereas high micronization temperature was needed with low seed moisture content in order to obtain the bologna which had comparable acceptability of flavour, overall texture, overall juiciness, and overall acceptability scores to bologna prepared with wheat flour, the industry standard for binders in bologna.

7. OVERALL CONCLUSION

The current thesis research was conducted to assess the potential for using chickpea flour as a binder in a low-fat pork bologna product. Bologna treatments in the entire project was formulated to produce a final composition of 11% protein and 10.0% fat, according to study 2. Chickpea flour was added at the level of 5% a dry basis. Bologna without binders and those with wheat flour (industry standard) served as controls. Findings indicated that both seed tempering moisture and micronization temperatures used impacted the physicochemical properties of the chickpea flours. Micronizing at 22% seed moisture resulted in flour with a dark yellow colour. However, having 22% seed moisture during heating is important to improve the functionality of chickpea flour (e.g., WHC, OAC, gelatinized starch content). Micronizing seeds with excess moisture leads to a presence of gelatinized starch, where the percentage of gelatinized starch content was found to increase as the micronized temperatures were raised. Moreover exposure of the seeds to heat at sufficient moisture results in denaturation and unfolding of the proteins. These phenomena play an important role in water holding capacity and oil absorption capacity and pasting properties upon reheating of the chickpea flour. When the chickpea flours were incorporated into the model low-fat pork bologna, the ability of flour to bind water and oil, shown in the study 1 did not translate into improvments to the cook loss, expressible moisture and purge loss values. In contrast, bologna containing wheat flour showed the greatest WHC among all bologna treatments. The addition of chickpea flour into bologna led to less colour acceptability compared to those containing wheat flour which might be due to the yellower colour of the bologna containing chickpea flour, based on CIE system and sensory evaluation from trained panel study. The results from texture profile analysis (TPA) and torsion parameters showed that adding chickpea flour from seed tempered to 22% seed moisture with 115^{o}C micronized temperature provided a harder texture than those of bologna with several added chickpea flour treatments (especially, those from untempered seeds or those tempered to 15% seed moisture and micronized to 115^{o}C) which were comparable to bologna containing wheat flour. Similar results were found in both the trained and consumer panel studies. This study found that overall texture, overall juiciness and flavour of bologna produced with chickpea flour from seed micronized to 150^{o}C and chickpea flour from seed tempered to 22% seed moisture following 115^{o}C was more acceptable than bologna with chickpea flour from non-micronized seed, moreover these treatments were comparable to bologna containing wheat flour.

Overall, chickpea flour from micronized seed at sufficient seed moisture and heat generally improved the sensory properties similar to bologna produced from wheat flour and the control, or even better than bologna added wheat flour in term of flavour intensity, flavour desirability, and over acceptability. Micronized chickpea flour has potential as a functional binder in the meat applications due to its low cost, resulting in acceptable overall protein contents while producing lower cost meat products (complying with the protein source requirements of the CFIA), nutritional value (fiber and mineral content) and good functional properties (e.g., water/lipid holding, emulsification).

8. FUTURE STUDIES

The present research demonstrated the feasibility of using chickpea flour as a binder in low-fat pork bologna. Chickpea flour used in this study contained seed hull, which may have affected colour. Investigation of the effect of seed coat on the physiochemical properties on treated chickpea flour would be useful, especially since the seed coat is high in fibre which could have an effect on water holding capacity. Moreover, phenolic substances especially tannin which are mostly responsible for the brown colouring of seeds (Elias et al., 1979), could have an effect on colour of the final product. Emami & Tabil (2002) and Segev et al. (2011) stated that tannins are mostly located in the seed coat of Desi chickpea, these substances lead to unpleasant flavours and reduces the bioavailability of vitamins and minerals since tannins decrease protein digestibility and solubility leading to the inactivation of digestive enzymes. Therefore, the amount of polyphenols should be determined.

In order to understand more about the effect of seed tempering moisture and micronization conditions on chickpea flour, differential scanning calorimetry (DSC) analysis could be used to detect changes in seed components as a function of temperature. In legumes, DSC has been employed for detection of starch gelatinization, protein denaturation, and oil melting points (Nielsen et al., 1998). These will be beneficial for the physical characteristics of the end product such as water holding and texture properties.

Furthermore, since low-fat pork bologna is an emulsion-based gel, it would be beneficial to study the emulsifying properties of chickpea flour and the gelation properties of the meat-flour material in greater depth. Emulsion activity (EA) and emulsion stability (ES) can be used to evaluate the emulsifying properties of protein flours since EA provides information on how well the flour emulsifies oil, whereas ES measures the strength of the emulsion over time (Boye et al., 2010). Protein gelation can be determined by looking at least gelation concentration (LGC) which may be defined as the lowest concentration required to form a self-standing gel (Boye et al., 2010). These data may be related to the ability of fat retention and the ability of protein to form the network structure in meat systems which have an effect on textural and sensory attributes of the final product (McClements & Decker., 2008).

The addition of chickpea flour in this study was used at the level of 5% however, results showed that bologna containing chickpea flour from seed which was tempered and micronized at the appropriate condition had overall acceptability similar to bologna produced with wheat flour. To improve formulations, an optimization study using surface response methodology based on sensory properties such as texture, juiciness, and flavour attributes would be important to carry out to maximize consumer acceptability of the product since this study found that texture, juiciness, and flavour have a considerable effect on overall acceptability of bologna containing chickpea flour.

Moreover, it would be interesting to know the effect of seed tempering moisture and micronization on volatile compounds as it will have a direct effect on taste perception. The presence of aldehydes, ketones, and alcohols, which are volatile compounds, has been associated with 'beany' aroma and off-flavor in legumes seeds (Shariati-Ievari, 2013). Many literature reports demonstrate that heat treatments decrease the level of volatile compounds (Iassonova et al., 2009; Žilić et al., 2010; Shariati-Ievari, 2013). Type of volatile compounds can be identified and quantified using a gas chromatography-mass spectrometry (GC-MS) system.

Legumes contain unsaturated fatty acids that are susceptible to oxidative deterioration resulting in the development of off-flavours. Lipoxygenase activity was done in this study with the flour stored for 2 years, therefore measuring lipoxygenase activity in fresh ground flour is needed since lipoxygenase activity can be decreased due to the storage time. Moreover, future study is also needed to identify the microbial stability of the bologna in order to determine the shelf-life of the products.

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Appendix A. Scorecard for trained panel study

Name:_____________________________

Date: ___________________

Instruction: Please evaluate the samples **colour** in the order that the sample # are arranged. **<u>Circle</u>** the descriptor that best describes your impression. Feel free to provide any comments as well.

Sample #	SCORE							
	8	**7**	**6**	**5**	**4**	**3**	**2**	**1**
REF	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
349	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
183	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
746	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
289	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
412	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown
605	Extremely pinkish red	Very pinkish red	Moderately pinkish red	Slightly pinkish red	Slightly brown	Moderately brown	Very brown	Extremely brown

Comments:

____

____**Thank you for your cooperation.**

Name:______________________________

Date: _________________

Sample No.:

Instruction: Please evaluate the samples in the order that the scorecards are arranged. For each characteristic, **circle** the descriptor that best describes your impression. Feel free to provide any comments as well. Please take a drink of water before beginning and between samples. Unsalted crackers are also available as needed.

DESCRIPTORS	**SCORE**							
	8	**7**	**6**	**5**	**4**	**3**	**2**	**1**
Texture: Initial firmness	Extremely firm	Very firm	Moderately firm	Slightly firm	Slightly soft	Moderately soft	Very soft	Extremely soft
Chewiness	Extremely hard to chew	Very hard to chew	Moderately hard to chew	Slightly hard to chew	Slightly easy to chew	Moderately easy to chew	Very easy to chew	Extremely easy to chew
Overall juiciness	Extremely juicy	Very juicy	Moderately juicy	Slightly juicy	Slightly dry	Moderately dry	Very dry	Extremely dry
Flavour: Saltiness	Extremely salty	Very salty	Moderately salty	Slightly salty	Very slightly salty	Not detectable		
Flavour intensity	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly bland	Moderately bland	Very bland	Extremely bland
Flavour desirability	Extremely desirable	Very desirable	Moderately desirable	Slightly desirable	Slightly undesirable	Moderately undesirable	Very undesirable	Extremely undesirable
Foreign flavour	Extremely intense	Very intense	Moderately intense	Slightly intense	Slightly weak	Moderately weak	Very weak	No foreign flavour
Overall acceptability	Extremely acceptable	Very acceptable	Moderately acceptable	Slightly acceptable	Slightly unacceptable	Moderately unacceptable	Very unacceptable	Extremely unacceptable

Comments:

____

____ **Thank you for your cooperation.**

Appendix B. Scorecard for consumer study

Consumer Sensory Evaluation Form for Bologna

Participant #: ___________.

Information

- This study is aimed to test the acceptability of pork bologna made with added chickpea or other flours.
- Make sure to fill out the demographic and product information section at the beginning of the study
- PLEASE DO NOT PATICIPATE IN THE STUDY IF YOU HAVE ANY FOOD ALLERGIES OR SENSITIVITIES.
- Make sure to grab your treat before leave!
- Thank you very much for your participation.

PART 1: Demographic information

The following questions are intended to understand the general demographic of participants. The information will be kept confidential and will only be used to understand broad trends, and not on an individual level. Please mark (X) appropriate boxes.

How many people live in your home including yourself?
Enter number: ___________.

How many children (<18 years old) live in your home?
Enter number: ___________.

Which of the following categories best describes your role in grocery shopping for your household?

☐ Primary shopper
☐ Share the shopping
☐ Someone else is the primary shopper

Which one of the following best describes your annual household income level before taxes?

☐ Under $20,000
☐ $20,000 - 39,000
☐ $40,000 - 59,000
☐ $60,000 - 79,000
☐ $80,000 - 99,000
☐ Over $100,000

Gender

☐ Male
☐ Female

--Continue to Next page--

Age Category	☐	Lower 19 years
	☐	20 - 29 years
	☐	30 - 39 years
	☐	40 - 49 years
	☐	50 - 59 years
	☐	60 - 69 years
	☐	Over 70 years
Education (Highest level Completed)	☐	Some Grade School
	☐	Some High School
	☐	High School Graduate
	☐	Some University
	☐	University/College Graduate
	☐	Graduate School
Ethnic background (Check all that apply)	☐	European
	☐	North American
	☐	First Nation
	☐	Asian
	☐	African
	☐	Central/South American
	☐	Other

-- Continue to Next Page --

Pork Bologna Consumer Survey
Please answer the questions below. The information will be treated with strict confidence and you will not be asked to identify yourself on the survey.

On average, how often do you consume bologna?

- ☐ 3 - 5 times per week
- ☐ 1 - 2 times per week
- ☐ 1 - 2 times per month
- ☐ I don't eat bolognas typically

When purchasing bologna, which fat level would you typically purchase?

- ☐ Regular fat
- ☐ Low fat
- ☐ I don't buy bologna

When purchasing bologna, which salt level would you typically purchase?

- ☐ Regular salt
- ☐ Low salt
- ☐ I don't buy bologna

Which type of bologna do you prefer to purchase?

- ☐ Pork
- ☐ Beef
- ☐ Chicken
- ☐ Made from several meats
- ☐ Other:_____________

Please indicate how important the following features are to you when shopping for bolognas.

	Not at all important ←--------------------→ Extremely important					
	1	2	3	4	5	6
Price	☐	☐	☐	☐	☐	☐
Fat content	☐	☐	☐	☐	☐	☐
Salt content	☐	☐	☐	☐	☐	☐
Exclusion of additives	☐	☐	☐	☐	☐	☐
Variety of flavours	☐	☐	☐	☐	☐	☐
Made from a specific meat source, e.g. pork, beef, chicken	☐	☐	☐	☐	☐	☐
Gluten-free	☐	☐	☐	☐	☐	☐

Below is a list of statements relating to food purchasing habits and life style. For each, please indicate how much you agree or disagree on the scale provided.

	Completely Disagree ←---------------------→ Completely Agree					
	1	2	3	4	5	6
I consider myself very health conscious.	☐	☐	☐	☐	☐	☐
I exercise on a regular basis.	☐	☐	☐	☐	☐	☐
I regularly read nutritional labels on the food I purchase.	☐	☐	☐	☐	☐	☐
I will pay more for a food product if it is more nutritious than a cheaper alternative.	☐	☐	☐	☐	☐	☐
Price is the most important factor I consider when I buy meat products.	☐	☐	☐	☐	☐	☐
I prefer to buy the lower-fat version of a food product if it is available.	☐	☐	☐	☐	☐	☐
I will opt for the lower-fat version of a food product, even at the expense of lower flavour quality.	☐	☐	☐	☐	☐	☐
I believe that pork is a good source of nutrition.	☐	☐	☐	☐	☐	☐
I believe that chickpea is a good source of nutrition.	☐	☐	☐	☐	☐	☐

<u>PART 2</u>: Consumer Sensory Evaluation of Pork Bologna

Instruction: Please first evaluate each sample visually for colour and appearance then taste the samples in the order that the sample codes are presented to you. **Please take a bit of cracker and a drink of water before and between samples**. You can avoid swallowing samples after tasting, is preferred. A waste cup is provided.

Sample code: ____________

How much do you like/dislike the **colour** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **appearance** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **overall texture** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **overall juiciness** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

How much do you like/dislike the **flavour** of this sample?

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

Considering all the characteristics of these samples, indicate your **overall acceptability**

like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
☐	☐	☐	☐	☐	☐	☐	☐

Would you buy this product? (check one)

☐ Yes ☐ No

Comments:……………………………………………………………………………………

……………………………………………………………………………………………………

Your comment is highly appreciated. Thank you for your cooperation.